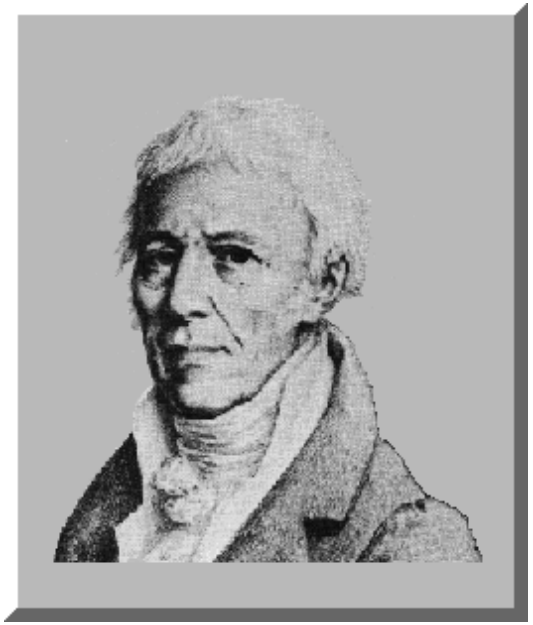
9. Eredità Epigenetica

Ruolo dell'eredità epigenetica

- Lamarck rivisitato
- Lamarck aveva torto nel pensare che l'ereditarietà dei caratteri acquisiti fosse il meccanismo principale dell'evoluzione (selezione naturale più comune)
- Tuttavia, ora sappiamo che l'ereditarietà dei caratteri acquisiti avviene, a volte, attraverso l'ereditarietà di modificazioni epigenetiche

Jean-Baptiste Lamarck (1744-1829)



Philosophie Zoologique, 1809
The environment drives heritable phenotypic change

Epigenetics will free us from the tyranny of DNA Mechanisms:
Histone Modification DNA methylation
Vive la révolution

Come il DNA controlla lo sviluppo di un organismo



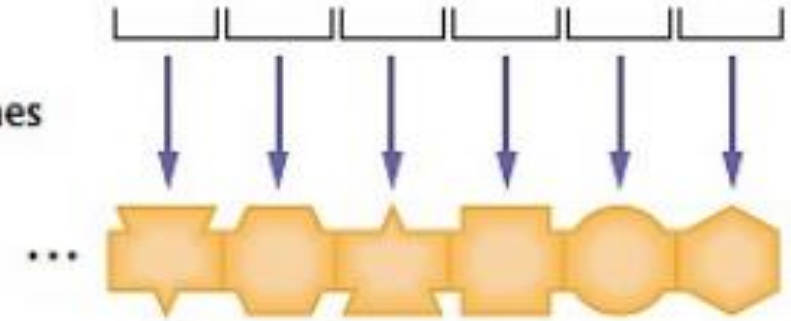
DNA
Self-replicating molecule

Each base determines one base of the RNA.



RNA
Copy of one strand of the DNA

A triplet of bases determines one amino acid.



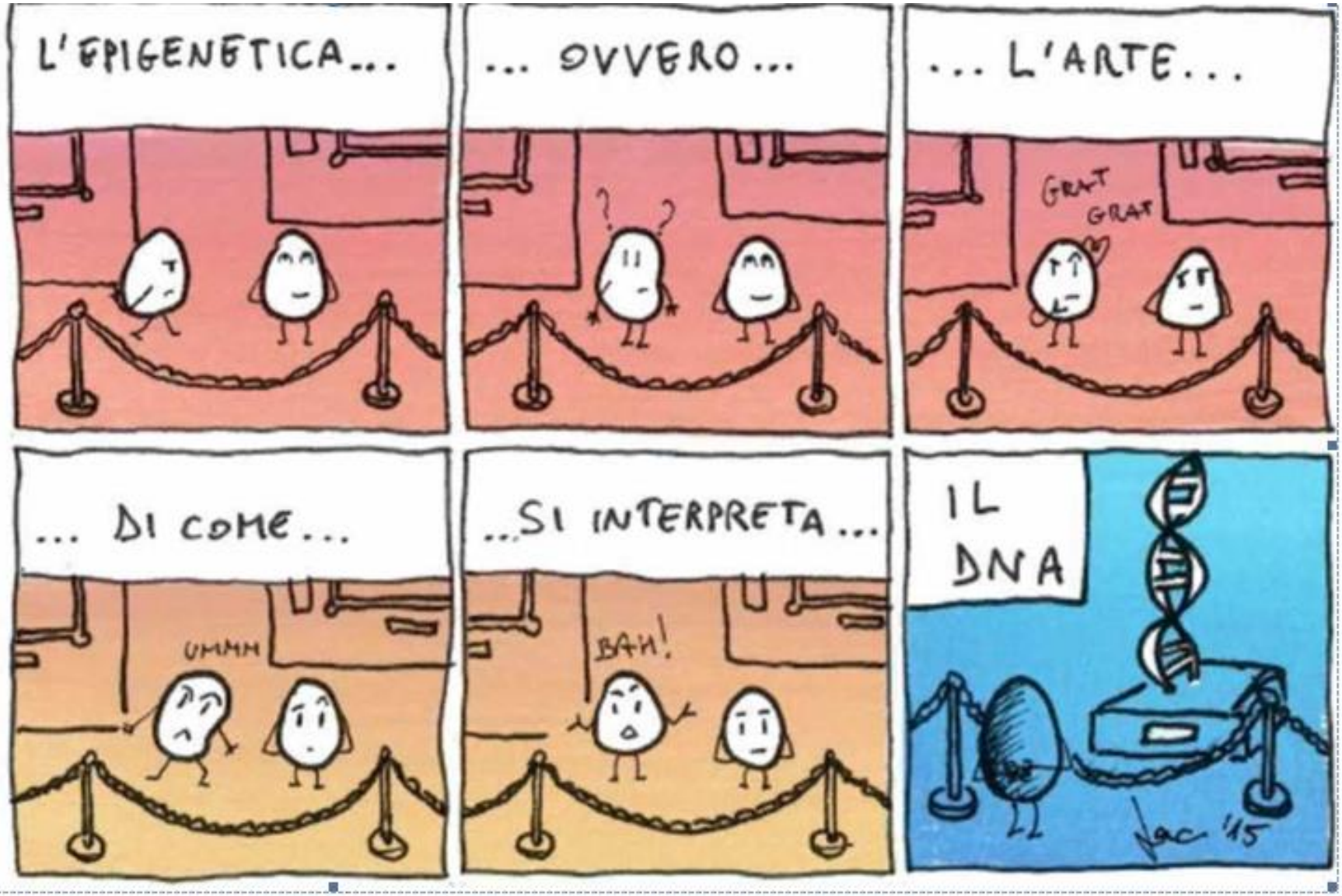
Protein
Some proteins become part of the body's structure. Others are enzymes that control the rate of chemical reactions.

Epigenetica

- Un ramo della genetica che è **connesso con variazioni del-l' espressione genica senza alcuna modificazione della sequenza del DNA**

- Alcuni geni sono attivi solo ad un certo stadio del ciclo vitale o ad un certo tempo del giorno, ecc
- Variazioni nell'espressione dei geni sono fondamentali per per i processi di apprendimento e memoria
- Differenze epigeneniche sono una spiegazione plausibile tra identici gemelli monozigoti

Al di sopra del DNA: ruolo dell'epigenetica nella regolazione dell'espressione genica



L’epigenetica permette all’evoluzione di aumentare la complessità biologica



Gene number, noise reduction and biological complexity

ADRIAN P. BIRD

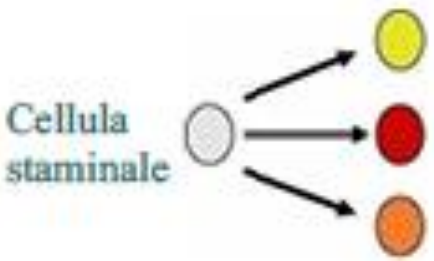
TIG MARCH 1995 VOL. 11 NO. 3

The above arguments suggest that the upper limit to the number of genes that an organism can tolerate is set by the ability of the prevailing biochemical machinery to distinguish essential gene expression from the background. Any advantage of having more genes is compensated, and ultimately may be outweighed, by increased logistical problems in distinguishing signal from noise.

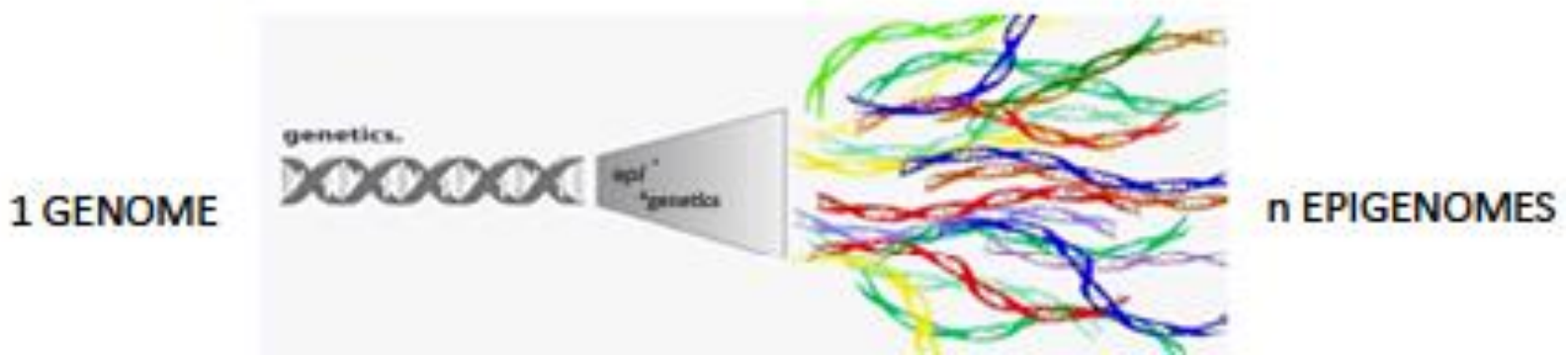
Bird (1995): L’epigenetica permette un controllo fine dell’espressione genica: cioè elimina il ‘rumore trascrizionale’ fornendo quel vantaggio per aumentare la complessità genomica (numero di geni)

1 genoma, n epigenomi

- il nostro corpo contiene **centinaia di diversi tipi cellulari** che, però, condividono la medesima origine.
- Man mano che le **cellule differenziano**, il loro **stato è governato dal silenziamento selettivo dell'espressione genica.**
- Questo silenziamento è per lo più **regolato da meccanismi epigenetici**
- questi **permettono di interpretare diversamente l'informazione contenuta nel D**NA. Errori di silenziamento possono produrre una pericolosa dissonanza.

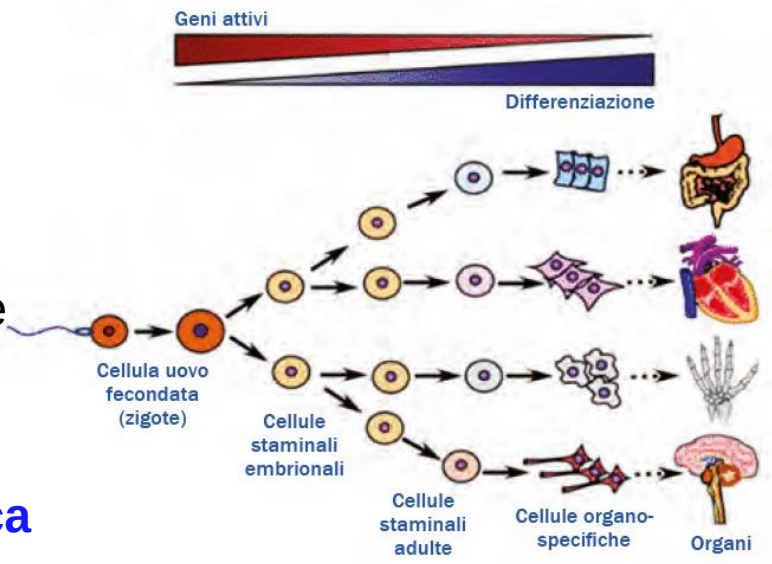






Cosa è l'Epigenetica?

- C.H. Waddington ha coniato il termine **epigenetica per indicare qualcosa al di là della genetica o in aggiunta alla genetica allo scopo di spiegare il differenziamento.**
- Come le differenti cellule staminali adulte conoscono il loro destino?
  - Mioblasti possono solo formare cellule muscolari
  - Cheratinociti formano solo cellule della pelle
  - Cellule ematopoietiche diventano globuli rossi
  - Ma tutte hanno la **stessa sequenza identica di DNA.**



Definizione moderna l'ereditarietà non è dipendente dalla sequenza

Processi cellulari di crescita e differenziamneto

- Come possono gemelli identici avere differenti colori naturali dei capelli?
- Come può un singolo individuo avere due differenti colori degli occhi?

Implicazioni:

- **Effetti epigenetici**: lo stesso genoma potrebbe esprimere diversi fenotipi: le differenze epigenetiche potrebbero comportare differenze fenotipiche anche tra gemelli identici (cloni)

Gemelli identici con differente colore dei capelli

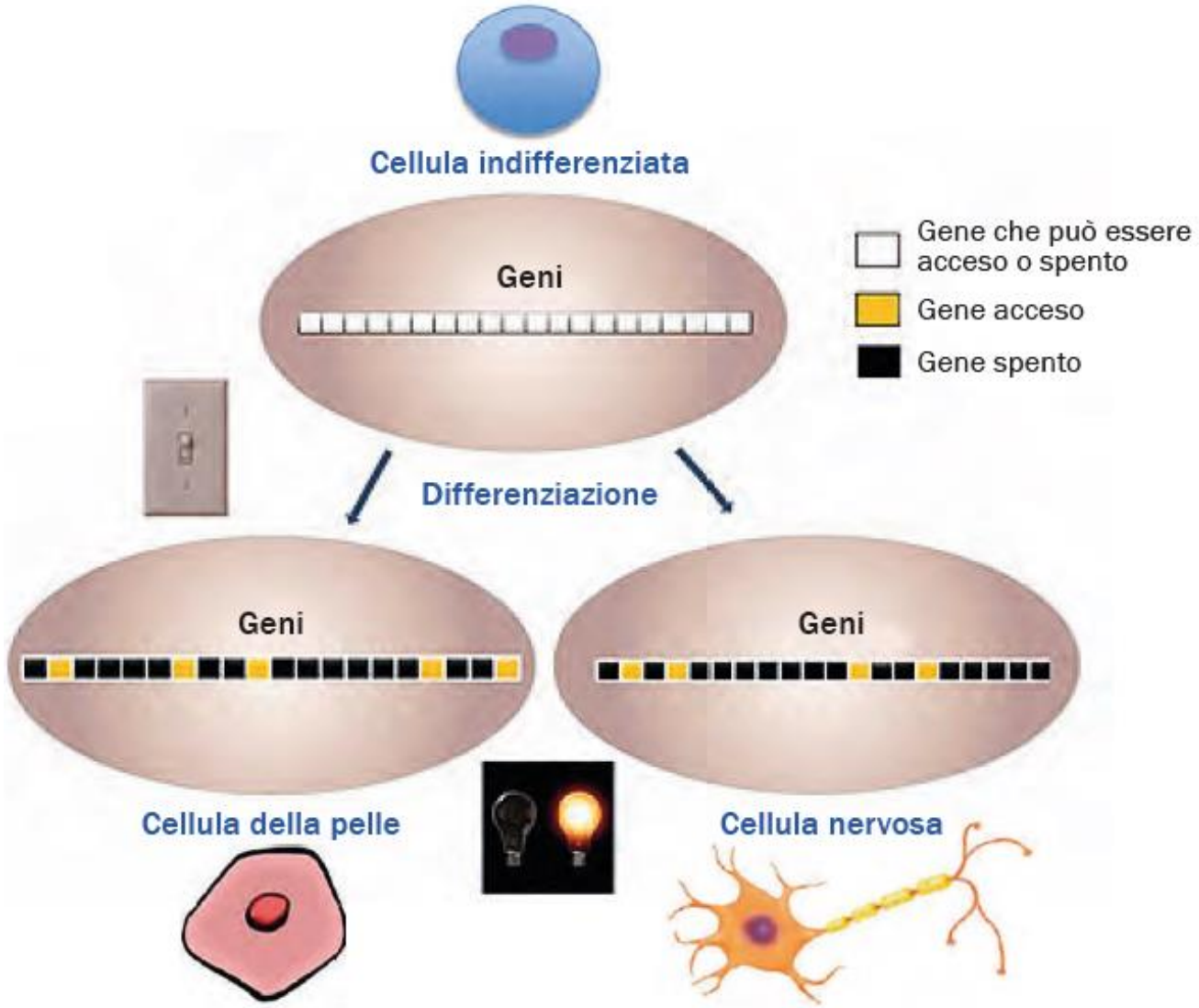


Un singolo occhio con due colori



- **Eredità epigenetica**: una evoluzione davvero rapida potrebbe avvenire senza cambiamenti nel codice genetico

Il processo di accensione e spegnimento genico durante il differenziamento cellulare



Epigenetic Modifications

- **"Epigenetic marks"** (methylation, etc.) are **made across the genome at each generation** to define cell types and patterns of gene expression in the developing embryo.
- These "**marks" define which genes** are **turned on and off.**
- **Marks from the previous generation** are typically removed **in the germline**, to enable totipotency of cells in early embryos

- Epigenetic changes could be environmentally induced

- **Occasionally the reprogramming is bypassed and some epigenetic marks get passed on → Lamarckian Evolution**

Modifiche Epigenetiche

- **“le marcature epigenetiche” (**metilazione, ecc**.)** sono attuate nel genoma ad ogni generazione per definire i tipi di cellule e i modelli di espressione genetica nell’embrione in via di sviluppo.
- Queste **“marcature”** definiscono **quali geni sono attivati e disattivati.**
- Le marcature della generazione precedente **sono generalmente rimosse nella linea germinale:** questo per consentire la totipotenza delle cellule nelle prime fasi embrionali.
- I cambiamenti epigenetici potrebbero essere indotti dall’ambiente.

- Occasionalmente la riprogrammazione è scavalcata e alcune marcature epigenetiche passano → **Evoluzione Lamarchiana**

(1) Chiarimento dei Concetti

- **Effetti Epigenetici**:
  - Tipicamente cambiamenti non ereditari nell'espressione genica o nel fenotipo cellulare causati da meccanismi diversi dai cambiamenti nella sequenza del DNA sottostante.
  - Esempio: metilazione del DNA e deacetilazione degli istoni: servono entrambi a sopprimere l'espressione genetica senza alterare la sequenza dei geni silenziati.
- **Effetti epigenetici transgenerazionali:** sono considerati **effetti sul fenotipo** (o sui modi di espressione genica) che:
  - sono rilevati in più di una generazione ,
  - non sono spiegabili con la genetica mendeliana (o da modifiche nella sequenza primaria del DNA).
  - Esempio: plasticità transgenerazionale, effetti materni.
- **Eredità epigenetica transgenerazionale**: il termine si riferisce agli effetti sul fenotipo (o sui modi di espressione genetica) che:
  - è trasmessa da una generazione all'altra da molecole nelle cellule germinali,
  - non può essere spiegata con la genetica mendeliana (o da modifiche nella sequenza primaria del DNA).

Modificazione Epigenetica (Effetto) *versus* Ereditarietà Epigenetica

- Se le **modifiche epigenetiche** si verificano da sole, NON sono un segno di **ereditarietà epigenetica**

- I cambiamenti che CONTANO per l'evoluzione, siano essi epigenetici o genetici (mutazioni),
  - **sono quelli che vengono trasmessi alla generazione successiva attraverso la linea germinale** (uova o sperma)

- Le modificazioni epigenetiche che si verificano in una singola generazione sono un tipo di **plasticità fenotipica.**

1. Phenotypic Plasticity

❑ Definition

- **Differences in phenotype that a genotype exhibits across a range of environments, because of changes in ge**ne expression

- **Changes in gene expression could be cause by environmental cues** instigating signal transduction...
  - changes in gene expression could also be caused by epigenetic modifications

- Some traits with a **plastic component**: intelligence, height, temperature tolerance, salinity tolerance, muscle mass…

1. Plasticità Fenotipica

❑ Definizione

- **Differenze nel fenotipo** che un genotipo mostra in una vasta gamma di ambienti, a causa di cambiamenti nell'espressione genica.

- **I cambiamenti nell'espressione genica potrebbero essere causati da segnali ambientali** che promuovono la trasduzione del segnale...
  - Potrebbero esserci anche cambiamenti nell'espressione genica causate da modificazioni epigenetiche
- Alcuni caratteri con una componente plastica: intelligenza, altezza, tolleranza alla temperatura, tolleranza alla salinità, massa muscolare…

Acclimation (≠ Adaptation)

1) Result of **Phenotypic Plasticity**

2) Not heritable (Not inherited)

3) Short term or developmental response within a single generation

4) Arises through **differential gene expression** or other regulatory mechanism **rather than natural selection**

Influenze ambientali su modelli di sviluppo

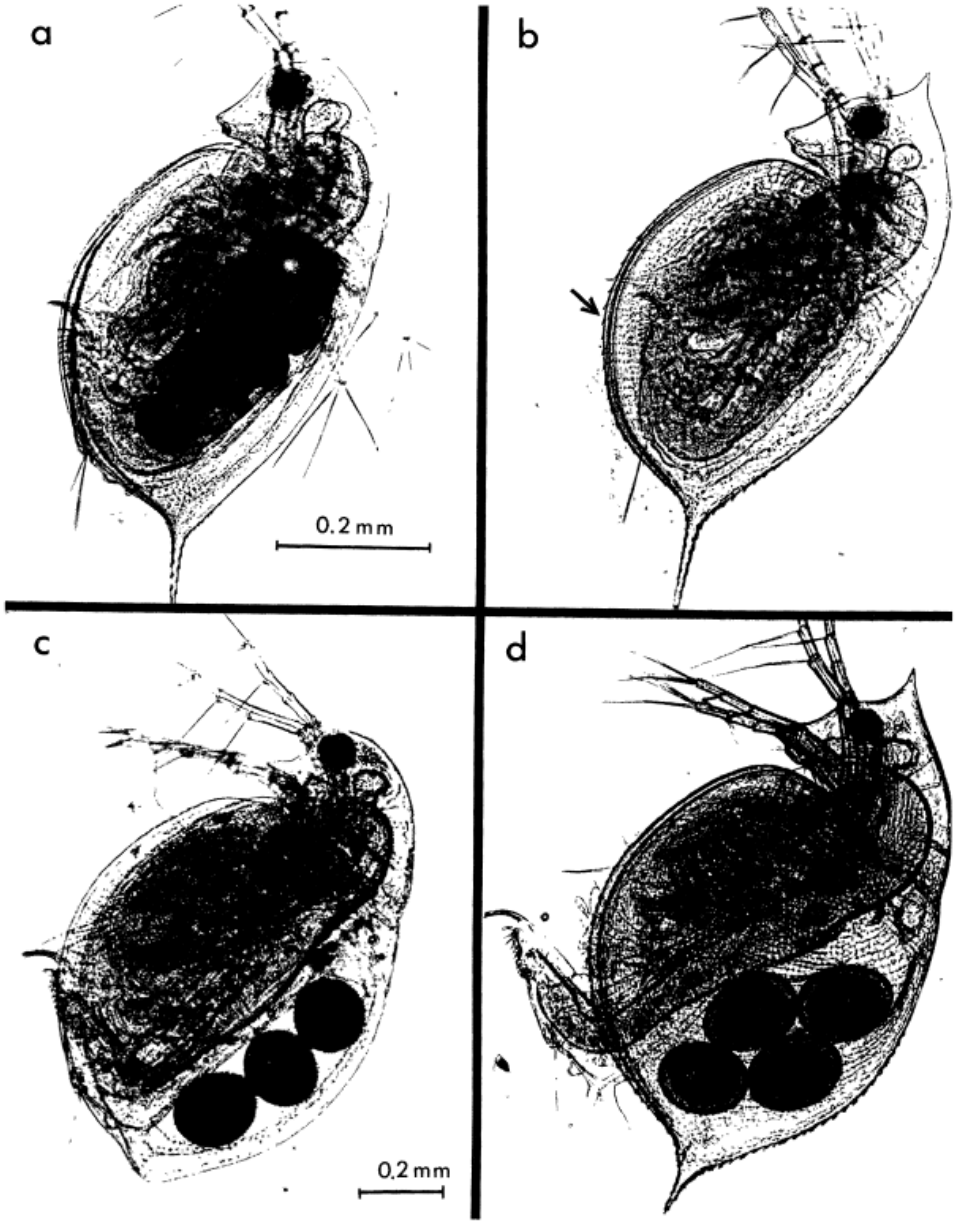


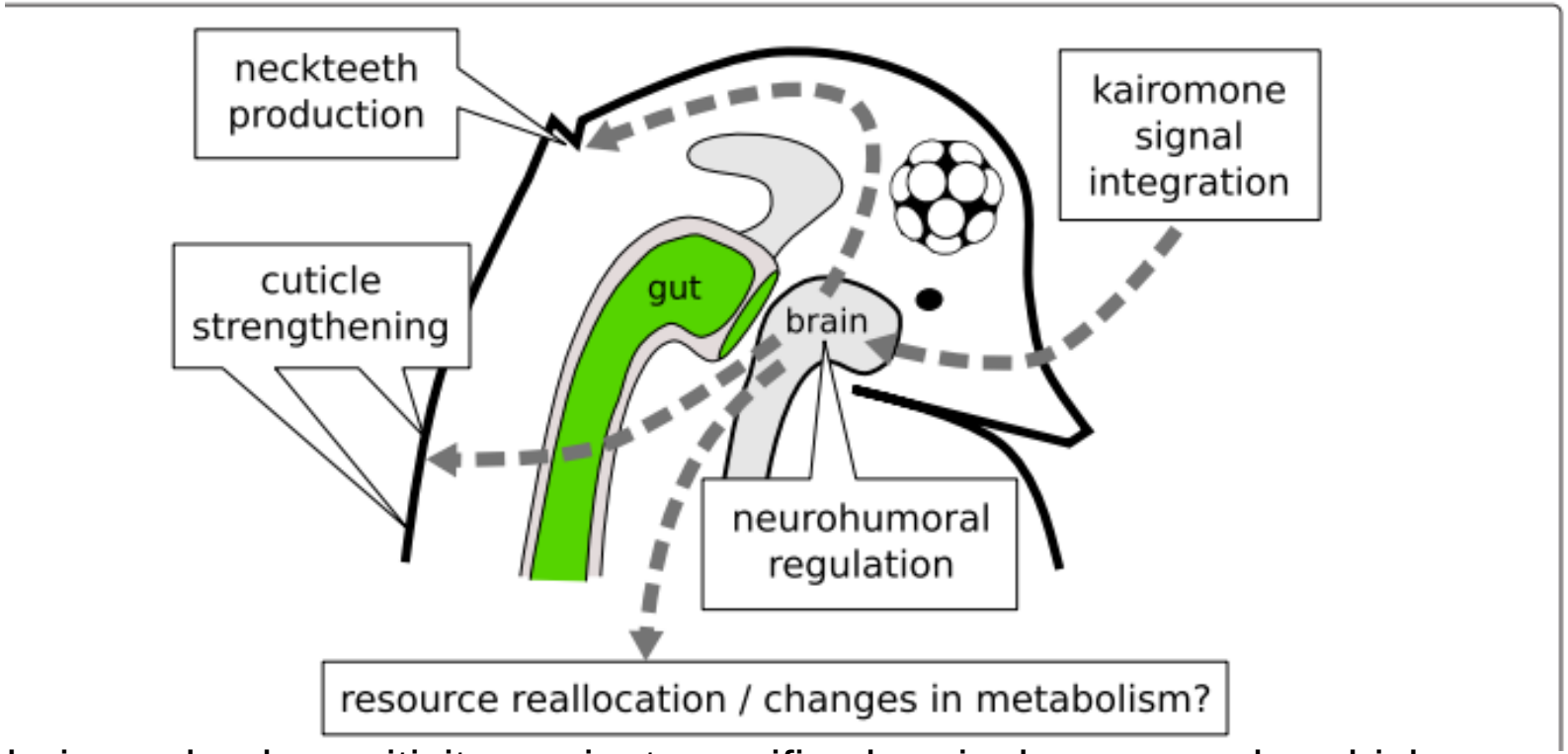
Fig. 2. Response of *Daphnia ambigua* to the *Chaoborus* factor. a. Maximum extent of helmet growth in 3rd instar control animals. b. Average helmet size in 3rd instar animals exposed to the *Chaoborus* factor. c. Maximum extent of helmet growth in control adults. d. Average helmet size in adults exposed to the *Chaoborus* factor. The arrow indicates the location of measurement of ventral spine length.

Predator induced formation of helmets in Daphnia

Daphnia **evolved sensitivity against specific chemical compounds,** which are unintentionally emitted by their predators(kairomnes)

Hebert and Grewe, 1985

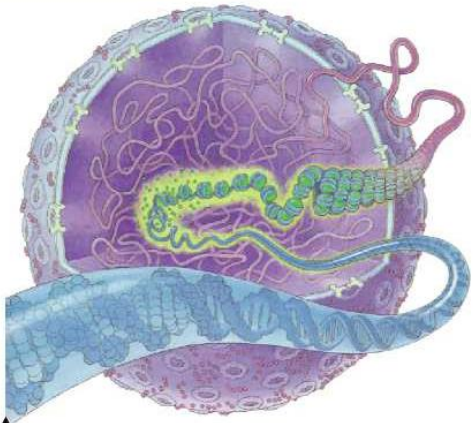
Presumptive scheme of physiological and morphological changes during kairomone-induction in D. pulex



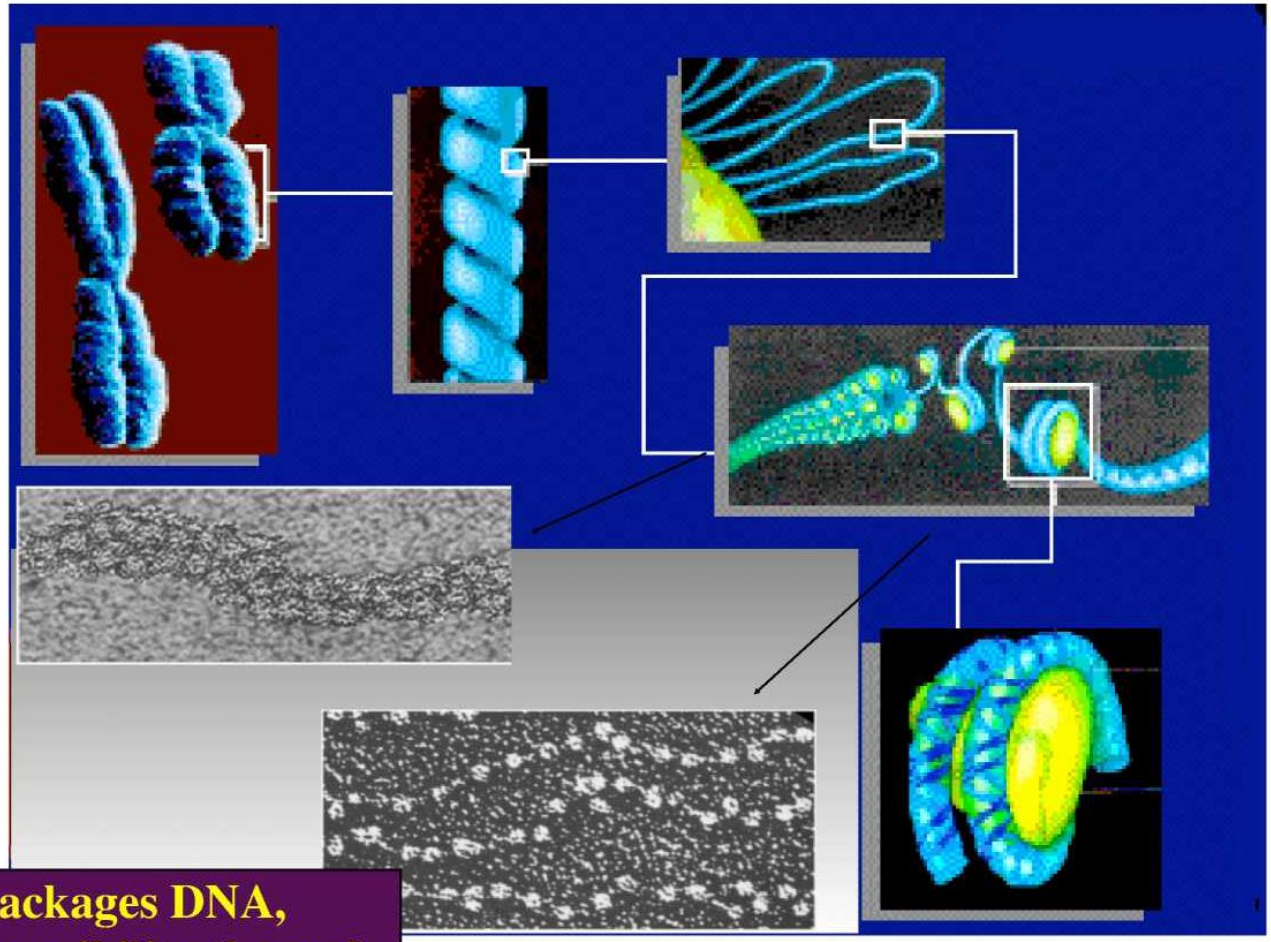
- Daphnia evolved sensitivity against specific chemical compounds, which are unintentionally emitted by their predators
- Daphnia species respond to environmental changes by developing different phenotypes given the same genetic background, a phenomenon of phenotypic plasticity.
- This means that the **genome must encode multiple developmental programs triggered by environmental conditions**.

(2) Modificazioni Epigenetiche

Packing of DNA into chromatin



2 m of DNA in a nucleus with a diameter of 5-10 μm



Chromatin not only packages DNA, but also regulates DNA accessibility through modifications in chromatin structure.

Livelli successivi di condensazione della cromatina dalla doppia elica al cromosoma metafasico in eucarioti

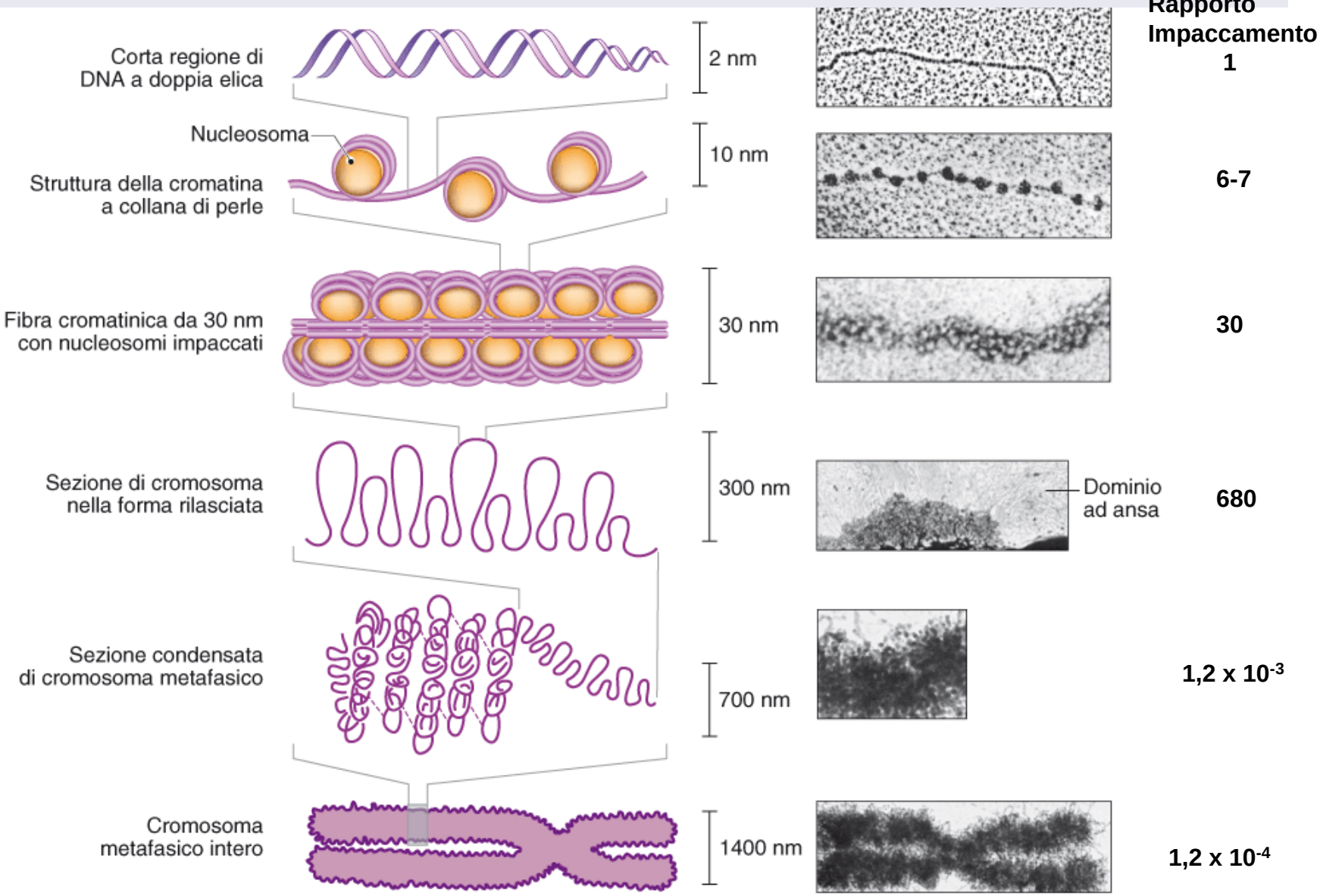
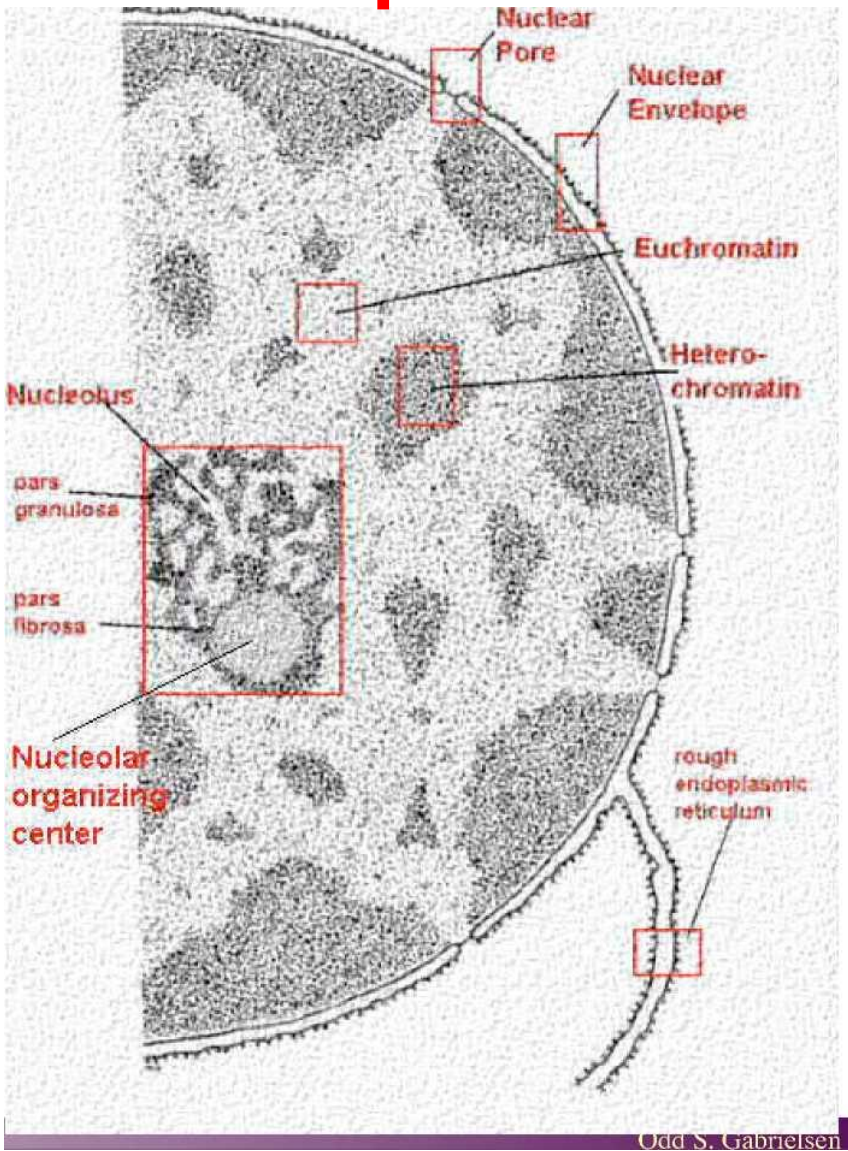


FIGURA 2.4 ► Livelli successivi di condensazione della cromatina dalla doppia elica al cromosoma metafasico.

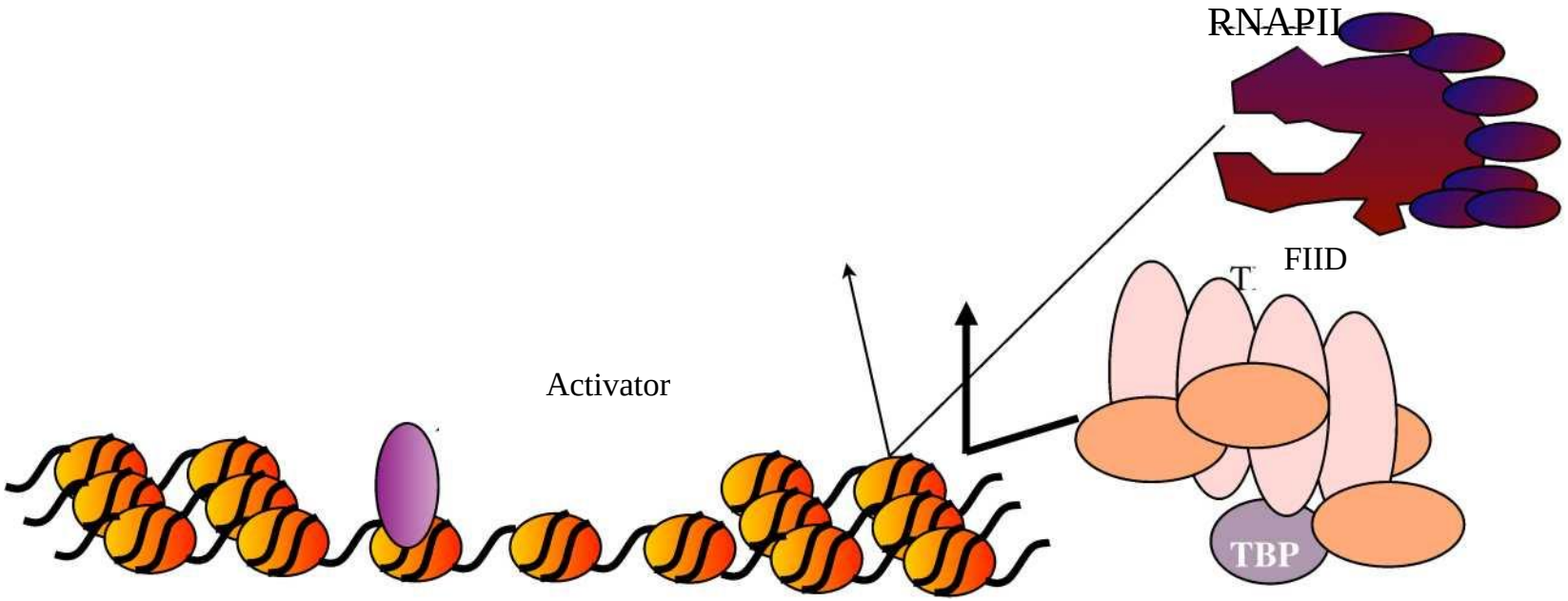
Chromatin compaction influences activity of DNA in transcription

- **Heterochromatin -** transcriptionally silent
- **Euchromatin -** transcriptionally active



Chromatin restricts transcription

- **Chromatin: a general inhibitor of protein access to DNA**
- **Packed chromatin will block access of basic RNApol II machinery to core promoters**
- **Chromatin needs to be unpacked, unwrapped or opened before transcription can take place**



Com'è regolata l'epigenetica?

- Almeno tre sistemi sono considerati elementi chiave per avviare e sostenere il cambiamento epigenetico:

- **metilazione del DNA;**
- **modificazione degli istoni :**
  - **acetilazione, deacetilazione**,
  - **livelli superiori di organizzazione della cromatina,**
  - **rimodellamento della cromatina**
- **RNA non codificanti** (ncRNA) associati con il silenziamento genico

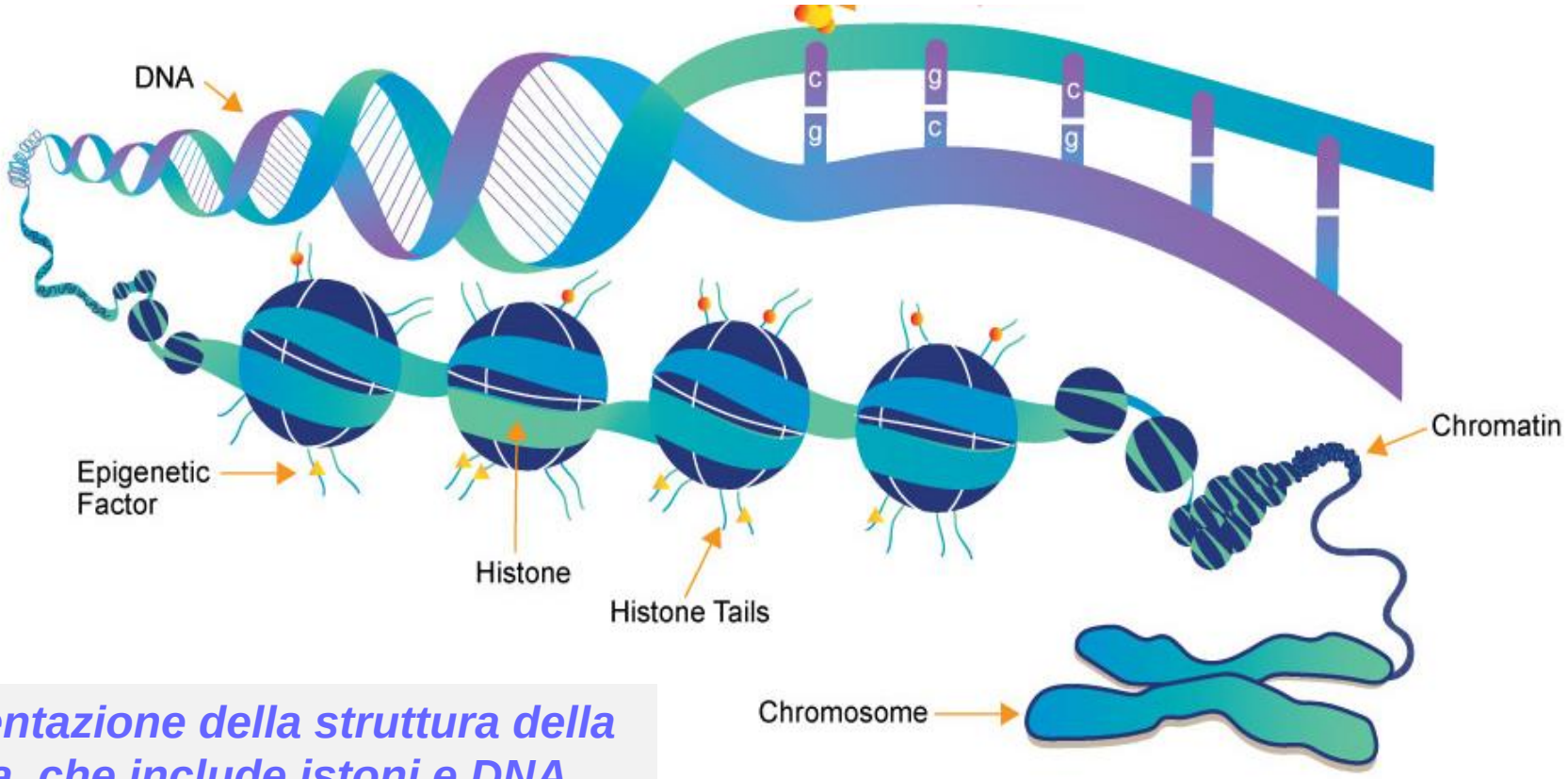
Esempi:

meccanismo basato su RNA interferente (RNAi): filamenti di RNA interferiscono con la trascrizione del DNA o traduzione dell'mRNA

- Frequentemente piccoli RNAs, spesso quelli che marcano elementi trasponibili
- sembrano agire principalmente su retrotrasposoni e altri elementi ripetuti.

Regolazione Epigenetica

- Tali sistemi lavorano spesso in maniera cooperativa, agendo insieme per **accendere o spegnere geni specifici** in un
  - determinato momento
  - o tipo cellulare.



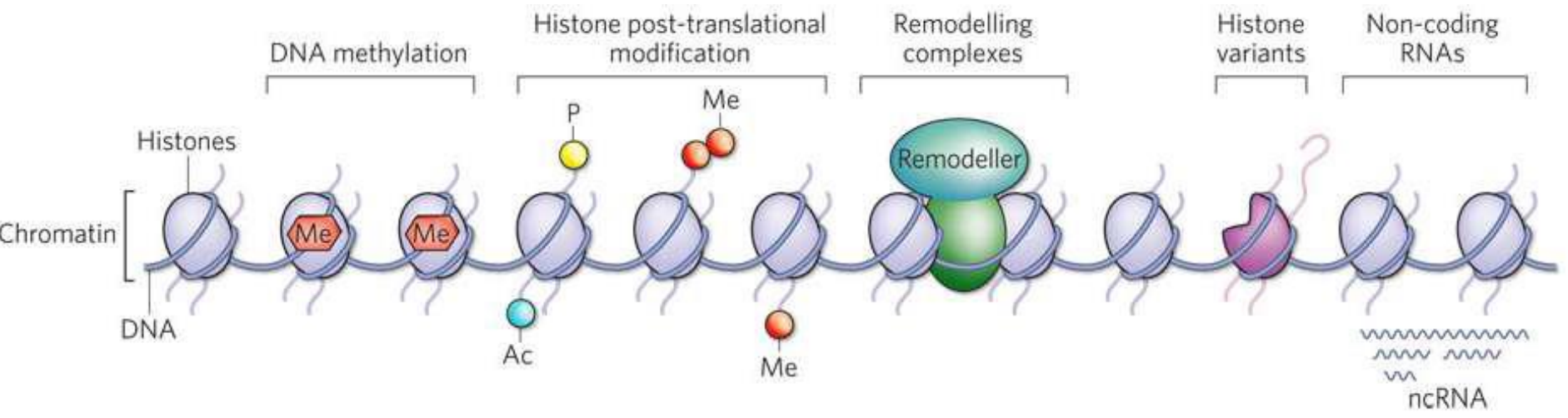
Rappresentazione della struttura della cromatina, che include istoni e DNA, suscettibili di marcature epigenetiche.

Meccanismi di Epigenetica

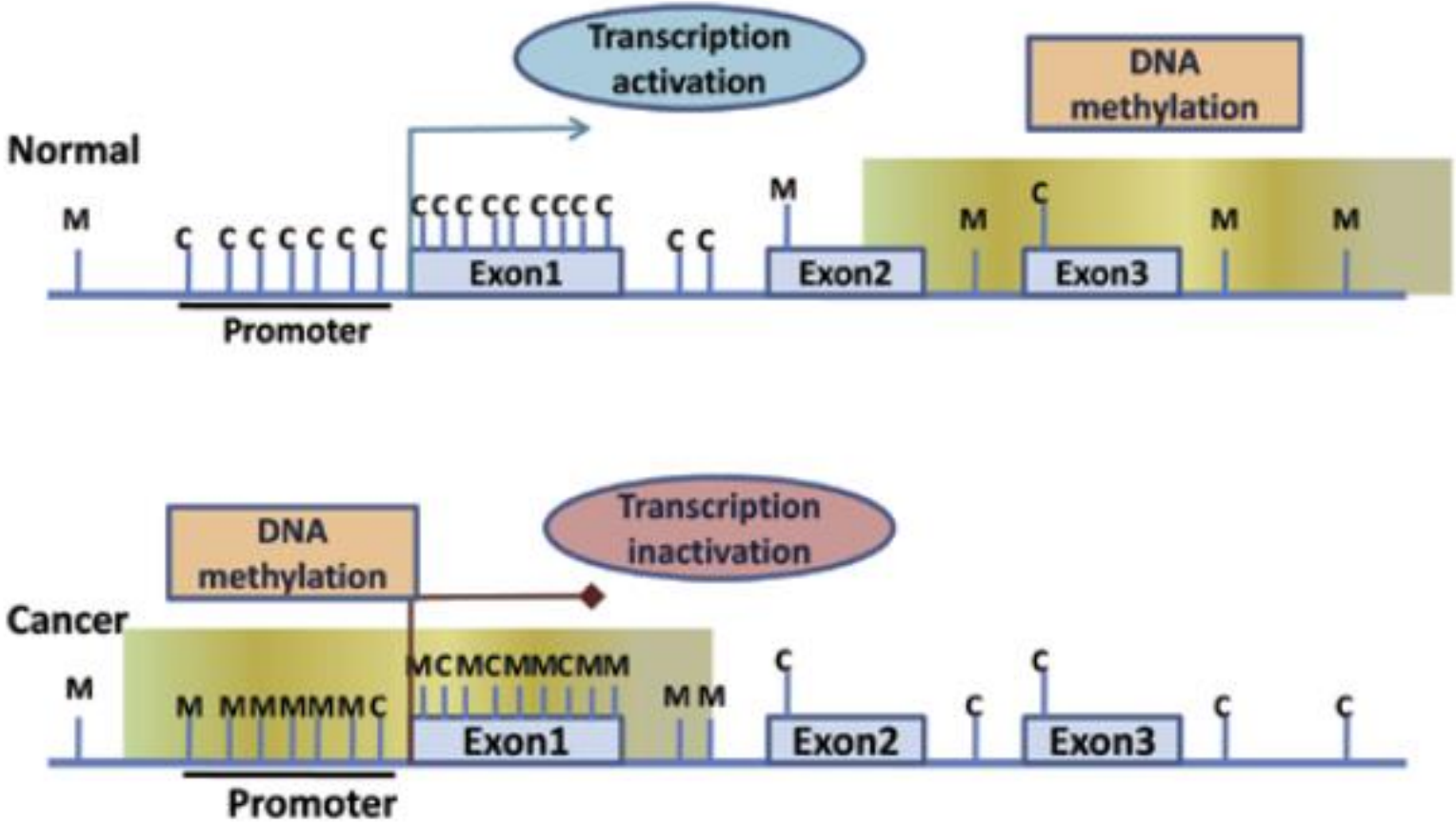
- **DNA Metilazione del DNA**
  - **RImodellamento della Cromatina & Modifica degli Istoni**
- **Via mediata da RNA Non-Codificante**

Codice Epigenetico:

- Consiste sia da **metilazione del DNA e modificadegli Istoni**
- **Codice genetico in ogni cellula è lo stesso**
- **Codice Epigenetico è tessuto- e cellula-specifico**



Global changes in DNA methylation in both normal and cancer cells.

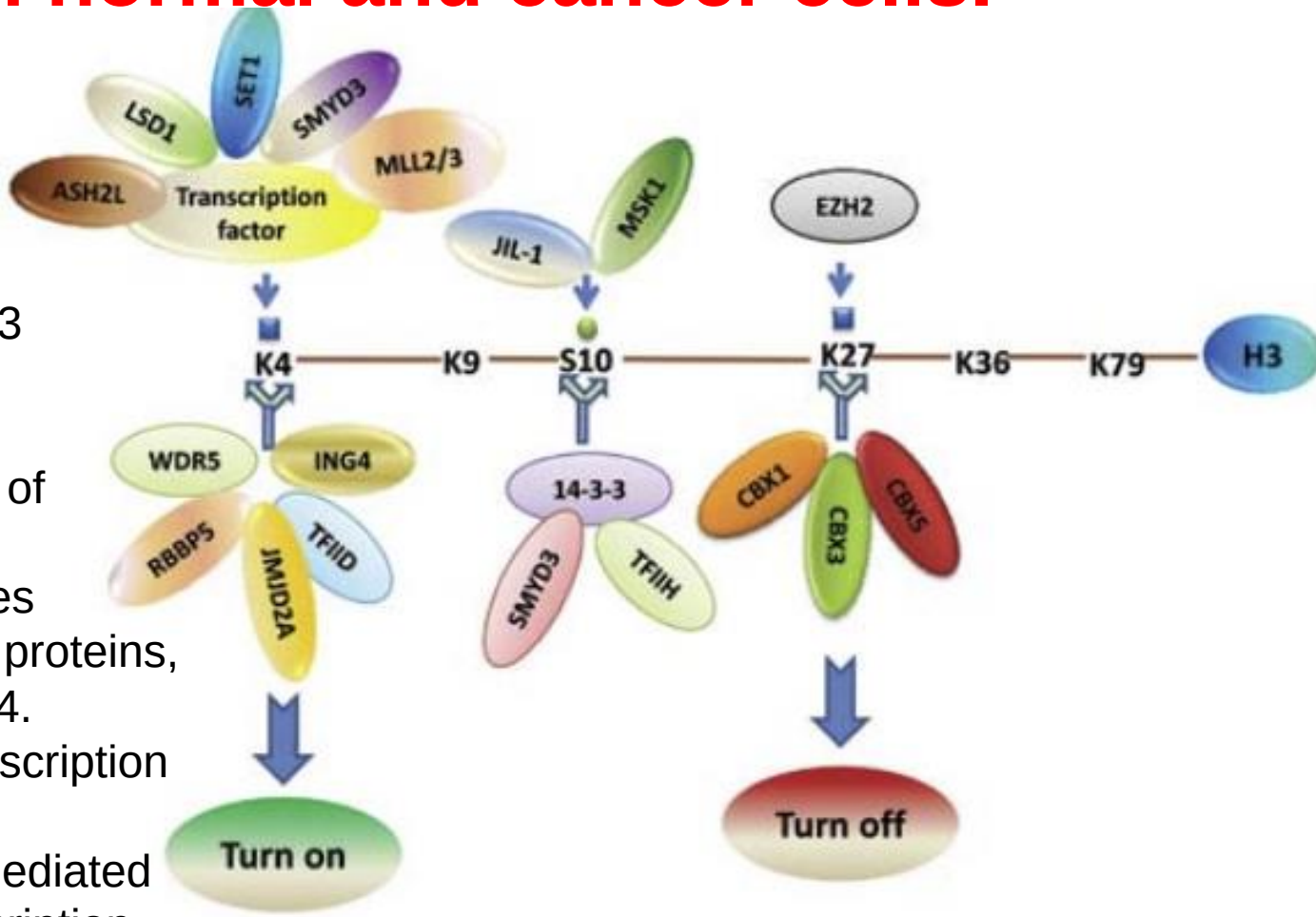


In normal cells, CpG islands in active promoters are not methylated, thus allowing transcriptional activation. CpG islands within coding regions are often methylated. Reverse patterns are observed in cancer cells.
C: Cytosine, M Metilated

Global changes in DNA methylation in both normal and cancer cells.

After TF binding to the gene promoter, a series of histone modification enzymes responsible for H3K4me3 are recruited to these nucleosomes, thus catalyzing trimethylation of histone H3 lysine 4. This modification provides binding sites for effector proteins, such as WDR5 and ING4. Subsequently, gene transcription is turned on.

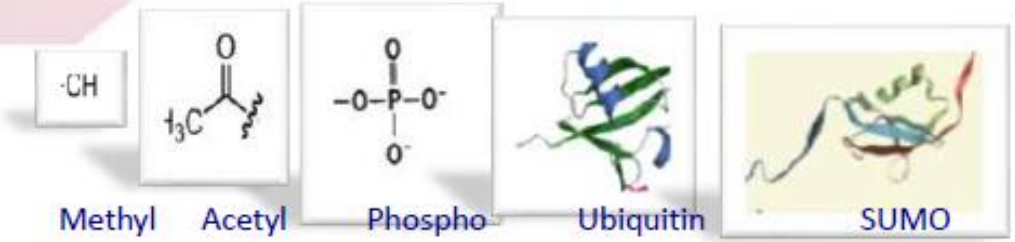
However, H3K27me3 mediated by EZH2 leads to transcription inactivation through interaction with CBX1, 3 and 5

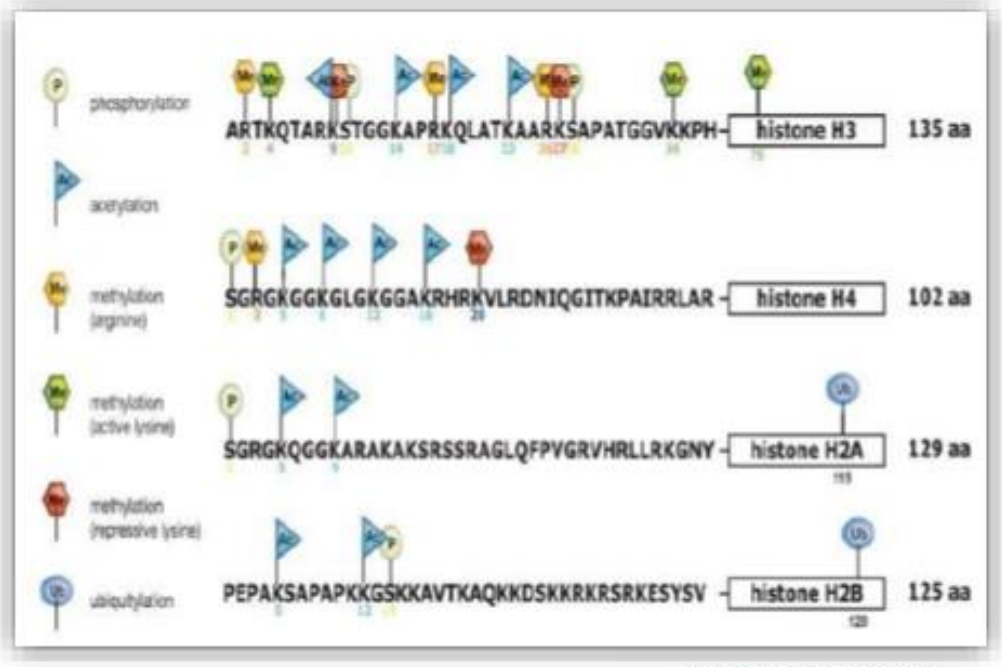


Types of Histone Modification

- **N-terminal tails of all histone**s are particularly of interest since they protrude out of the compact structure.
- These N-terminal tails are often subjected to a variety of post-translational modifications such as,

1. Acetylation
2. Methylation
3. Phosphorylation
4. Ubiquitination
5. Sumoylation
6. ADP ribosylation



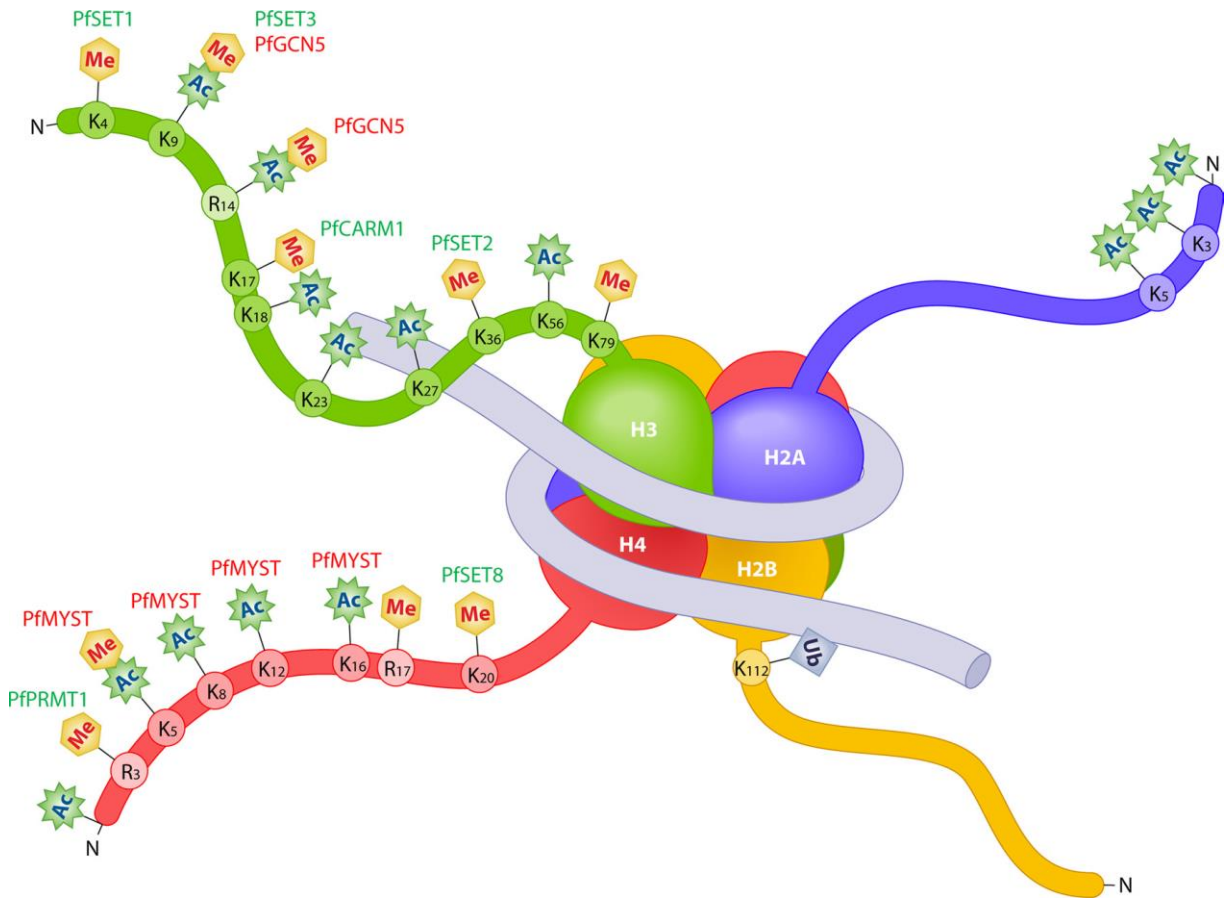


Bhaumik, Smith, and Shilatifard, 2007.

➢ It has been proposed that these modifications result in a '**code**' which can be read by proteins involved in gene expression and other DNA translations

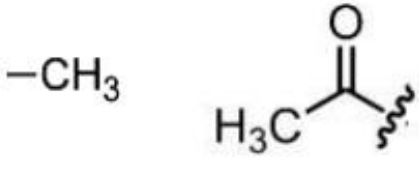
Schematic drawing of a nucleosome with the four canonical histones (H3, H4, H2A, and H2B) in *P. falciparum.*

- . The covalent PTMs of the histone tails (methylation [Me], acetylation [Ac], and ubiquitination [Ub]) and enzymes catalyzing the addition of the PTMs (PfGCN5,
- PfSET1, PfSET2, PfCARM1, PfPRMT1, PfMYST, and PfSET8) are highlighted. 1



Covalently attached groups (usually to histone tails)

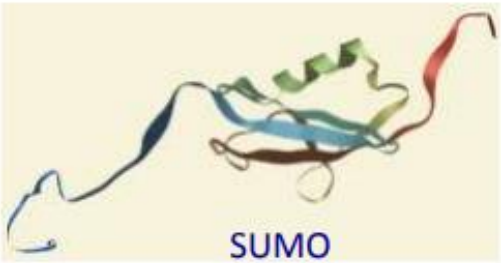
- Covalently attached groups (usually to histone tails)



Methyl Acetyl Phospho

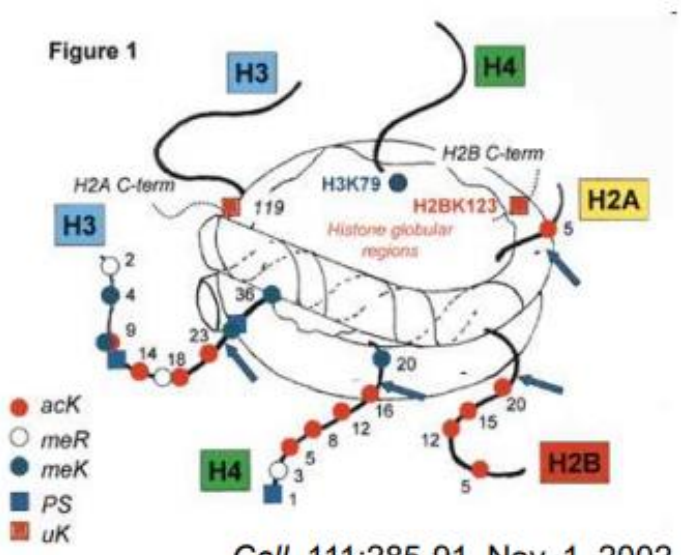


Ubiquitin



SUMO

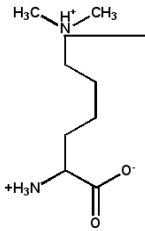
- Reversible and Dynamic
  - Enzymes that add/remove modification
  - Signals
- Have diverse biological functions



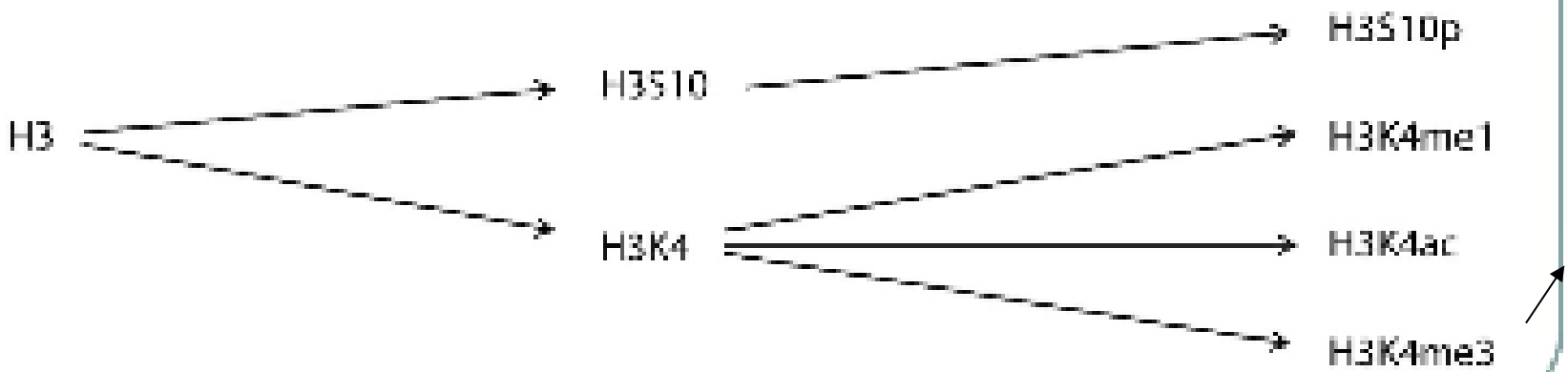
Cell, 111:285-91, Nov. 1, 2002

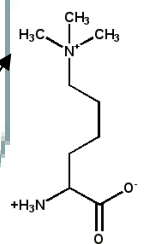
A schematic of short-form nomenclature for posttranslational histone modifications

Histone protein (isoform)	+	Amino acid code	+	modification
H3, H3.1, H3.3		K ... Lysine		p ... phospho
H4		R ... Arginine		ac ... acetyl
H2A, H2A.z, macroH2A		S ...Serine		me1 ... monomethyl
H2B		followed by position		me2 ... dimethyl me3 ... trimethyl

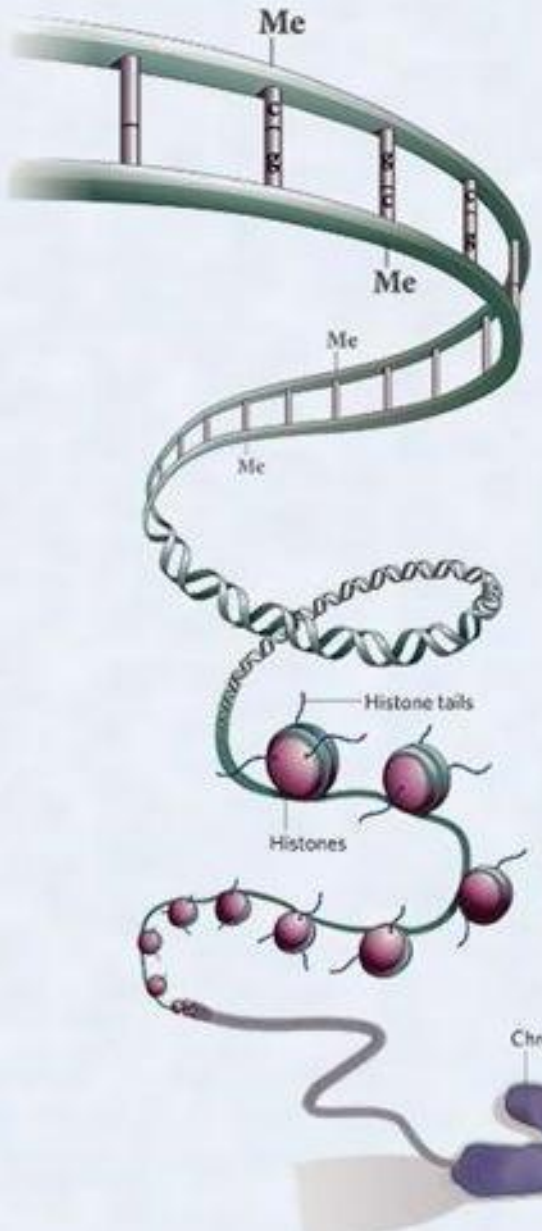


Examples





DNA Methylation & Histone Modifications Form the Epigenetic Code



The 'epigenetic' code

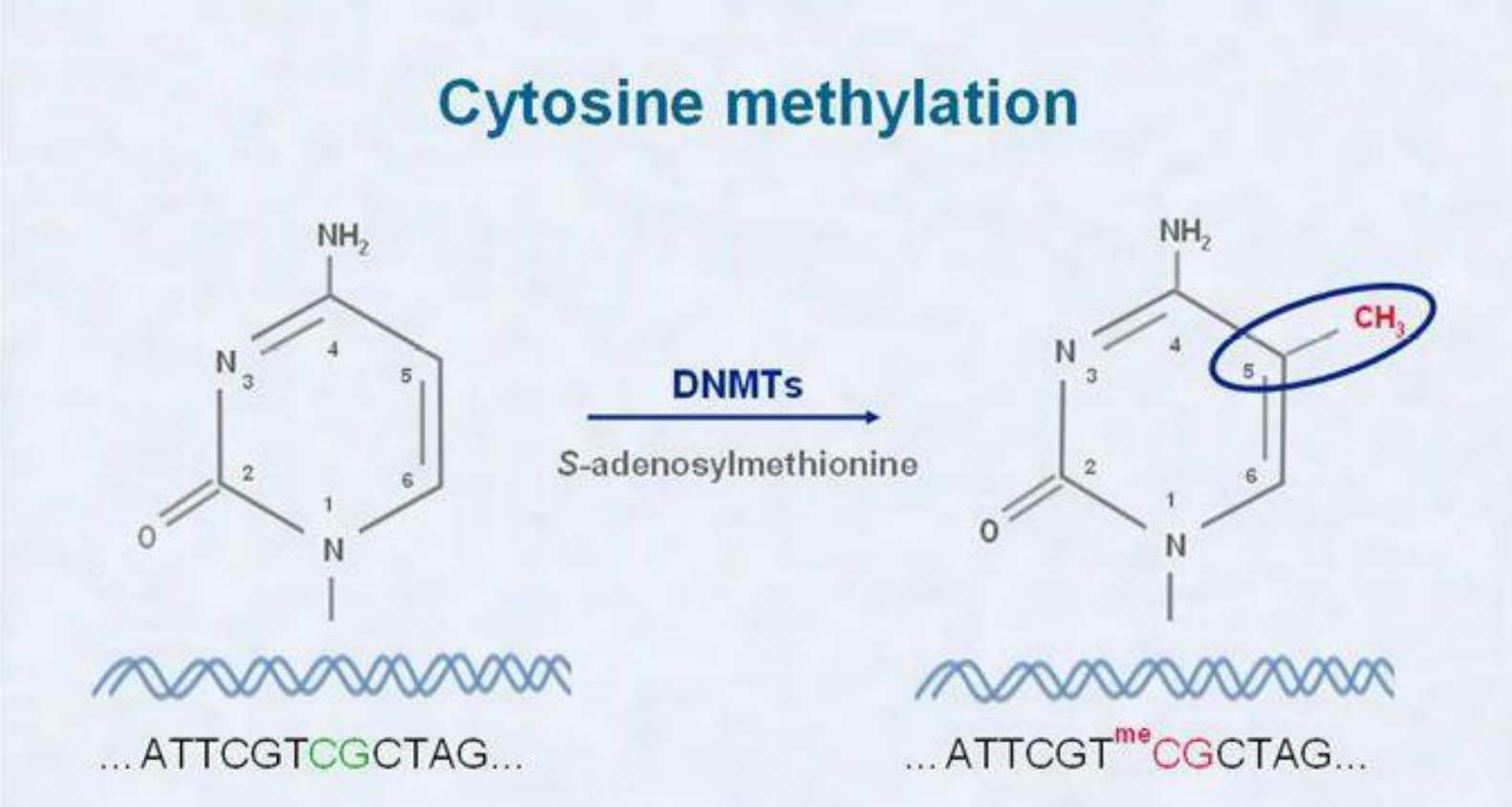
DNA methylation

Methyl marks added to certain **DNA bases repress gene activity**

Histone modification

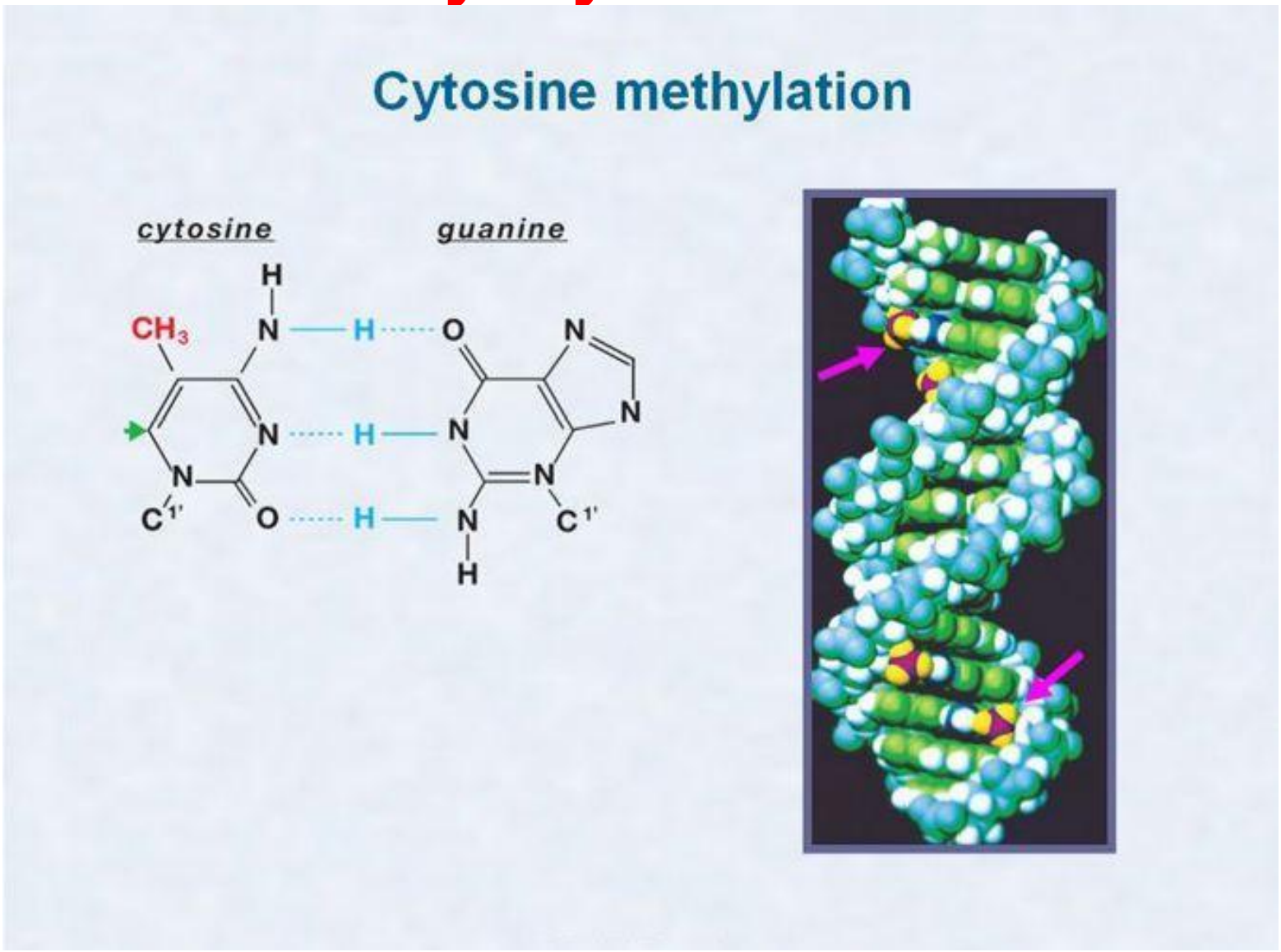
A combination of different molecules can attach to the 'tails 'of proteins, called histones. **These alter the activity of the DNA wrapped around them**

1. Methylation of Cytosine in DNA

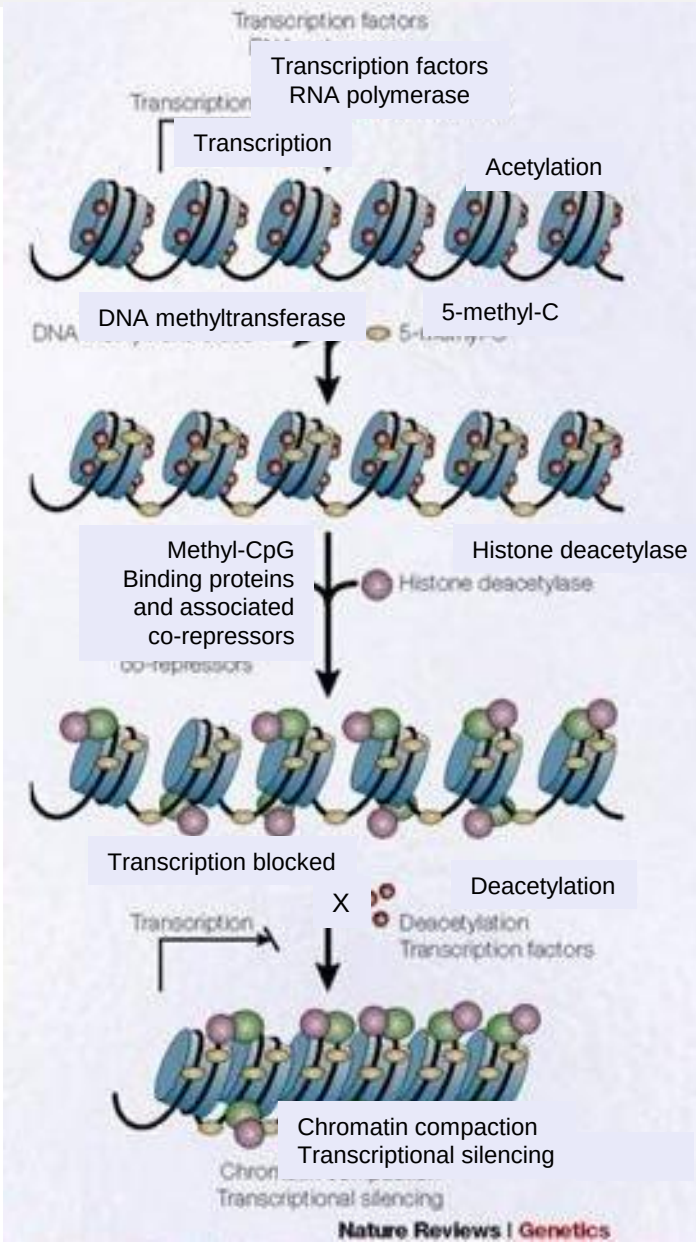


- La metilazione del DNA è la **modifica epigenetica più studiata.**
- La metilazione più abbondante nel DNA è **l'aggiunta di un gruppo metile al nucleotide citosina**, in particolare **nelle regioni del DNA**, dette **promotori**, dove inizia la trascrizione del gene a cui dovrà legarsi l'RNA polimerasi per iniziare la trascrizione.

5-Methyl Cytosine in DNA



Cytosine Methylation Maintains Inactive-Condensed Chromatin State



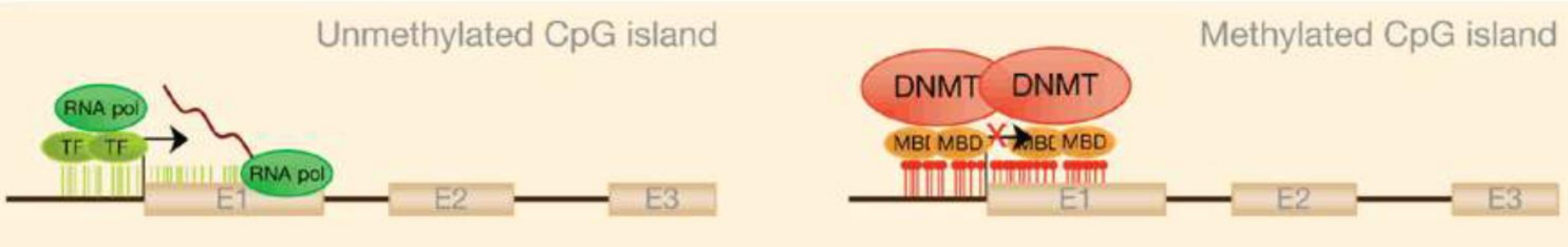


CpG Islands

- **CpG island**: a cluster of CpG residues often found near gene promoters (sequences ~1000 base pairs in length with a GC content more than 60%)

- ~29,000 CpG islands in human genome (~60% of all genes are associated with CpG islands)

Methylated CpG Islands Inhibit Transcription

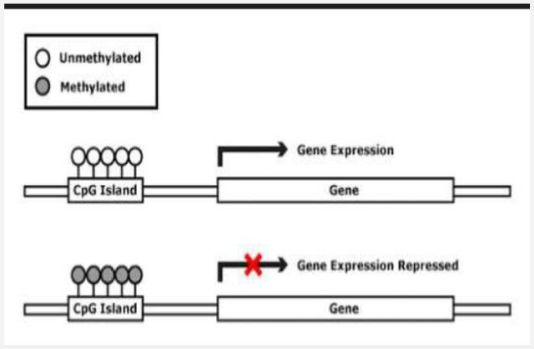


- **More than half of human promoters contain CpG islands**
- **Promoters are usually unmethylated**

REPRESSION OF CPG-DENSE PROMOTERS

- In humans about 60-70% of genes have a **CpG-islands** in their promoter region.
- DNA methylation of CpG-islands is linked **with transcriptional repression**.
- DNA methylation may effect transcription of genes in two ways :

1. DNA methylation effects the binding of transcriptional proteins to the gene.



2. Methylated DNA may binds with **methyl-CpG-binding domain proteins** (MBDs).

- They attract histone deacetylases and other chromatin remodelling proteins which modify histones forming compact inactive chromatin (heterochromatin).

In Plants

- **DNA methylation occurs at**
  - Symmetric CG -24%
  - Symmetric CHG -6.7% (H = A, C, or T)
  - Asymmetric CHH -1.7%

(Law et al., 2010)

DNA methylation

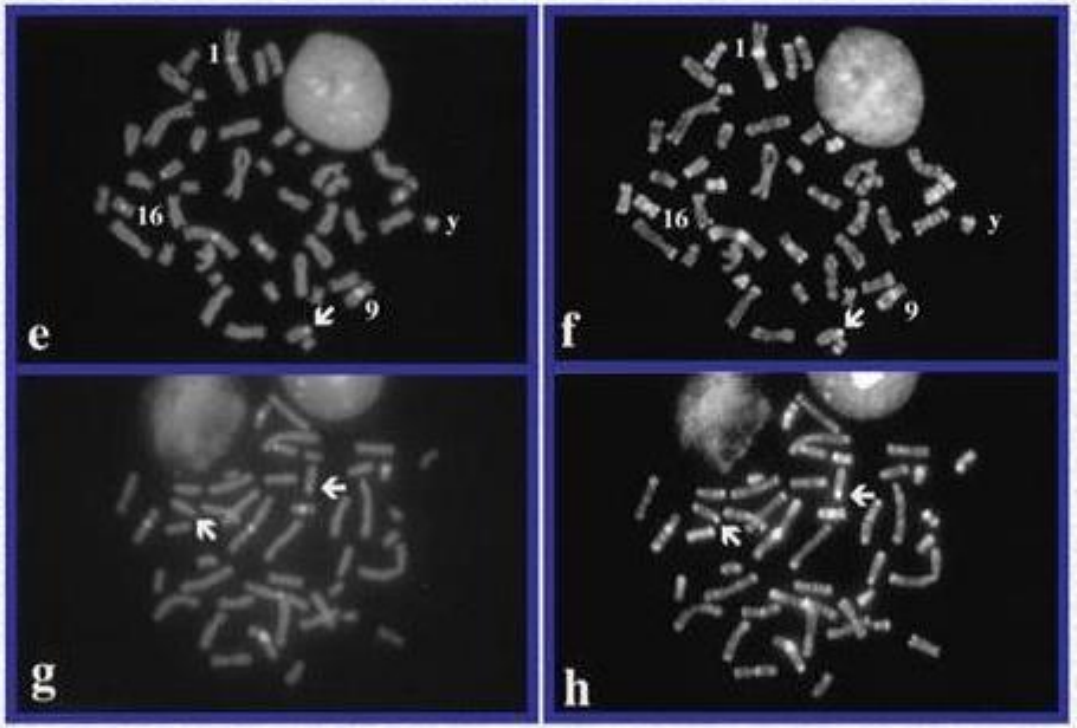
- **Tissue specific**
- **Time specific**
- **Unidirectional**
- **Normally permanent and stable**
- Amount of DNA methylation of cytosine differ in different tissue
- **Rate of cytosine methylation differ strongly between species**

-14.0 % in Arabidopsis
- 8.0 % in Mouse
- 2.3 % in *E. coli*
- 0.03 % in Drosophila

5-Methyl Cytosine is Found in Heterochromatic Regions

Distribution of C met in mammals

- Heterogeneity visible at cytogenetic scale
- Associated with heterochromatic regions

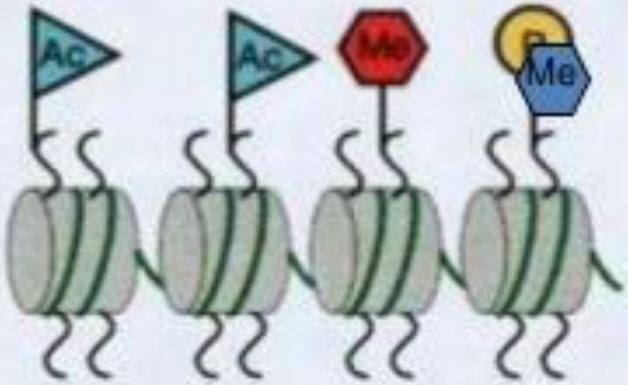


PMID: 9609658

Structure & Epigenetics of Euchromatin versus Heterochromatin

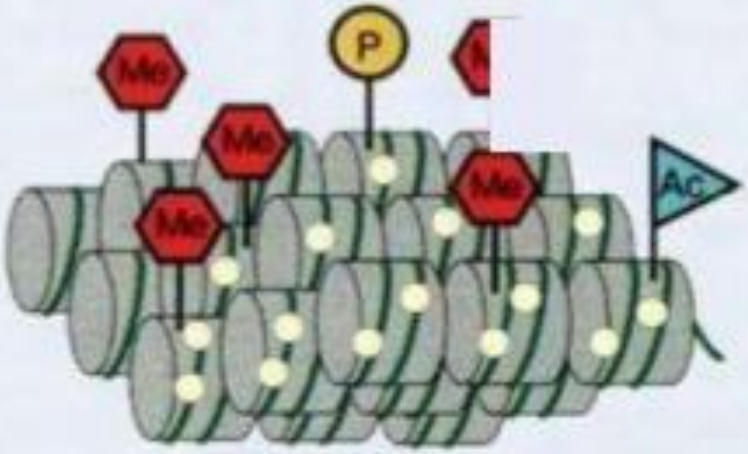
DNA methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials

Euchromatin



- High histone acetylation
- Low DNA methylation
- H3-K4 methylation

Heterochromatin



- Low histone acetylation
- Dense DNA methylation
- H3-K9 methylation

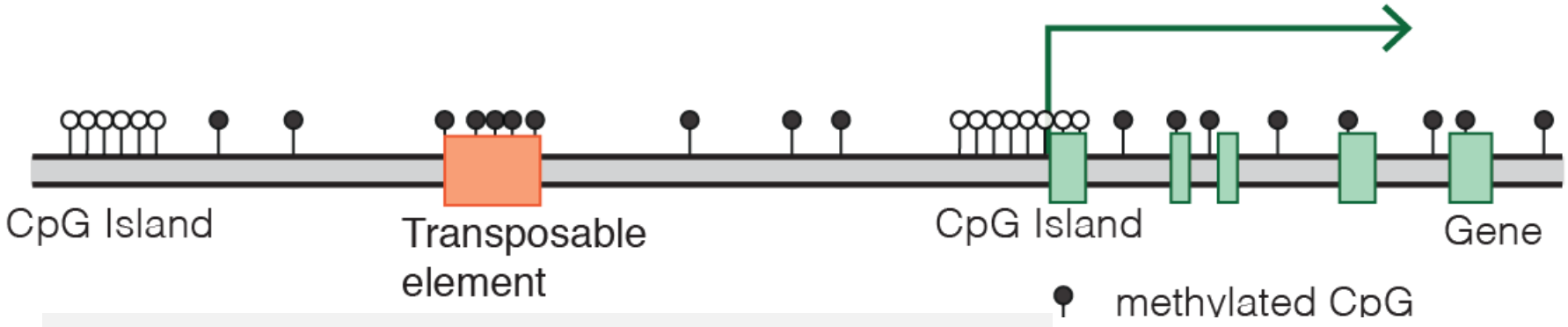
Le principali funzioni della metilazione sono collegate alla repressione della trascrizione:

- **Difesa contro i trasposoni**:

  la metilazione e' fondamentale per mantenere silenti i genomi dei trasposoni e dei retro-trasposoni

- **Regolazione genica:**

  la metilazione contribuisce a stabilire e mantenere uno stato trascrizionalmente inattivo (eterocromatina)



Typical mammalian DNA methylation landscape

DNA Methylation: Role

- **Crucial part of normal organismal development** and cellular differentiation in higher organisms
- **Alters gene expression** pattern in cells
- **Suppresses expression of viral genes** and other deleterious elements that have been incorporated into the genome of the host over time
- **Plays a crucial role in the development** of nearly all types **of cancer** (hyper-methylation, which represses transcription of the **promoter regions of tumor suppressor genes**)
- Might be the mechanism for long term memory storage

Enzymes in DNA methylation

❑ Plants

>30% of cytosines

- CG - DNA Methyltranferase 1 (MET 1)
- CHG – Chromomethylase 3 (CMT 3)
- CHH – de novo methylation (DRM)

❑ Animals and other eukaryotes

<10 % of all cytosines

- Only in CG dinucleotide
- Maintainance by DNA methyltransferase(DNMT)

(Niederhuth et al., 2014)

Enzymes involved in DNA methylation

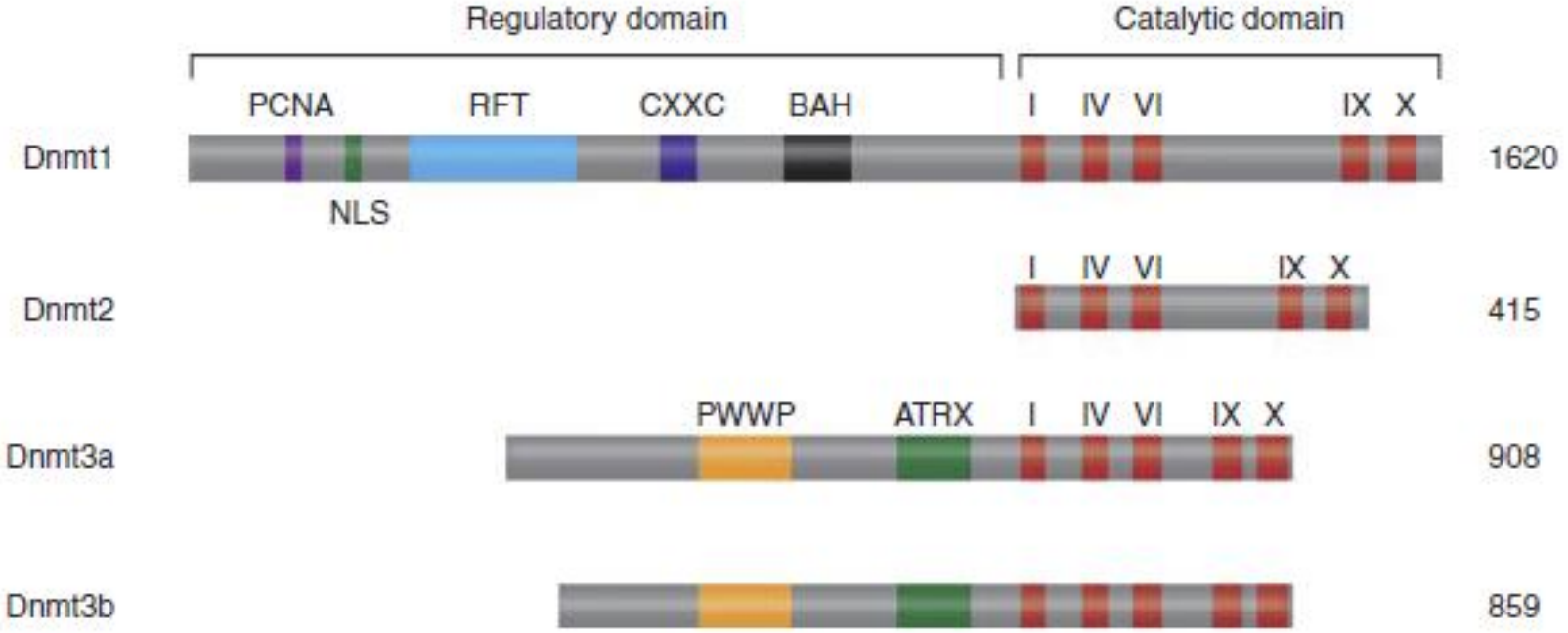
- Enzymes involved-

  DNA METHYLTRANSFERASES (DNMTs)

- **DNMTs** catalyze this reaction at different times during the cell cycle.
- In Mammals, there are 5 known methyl transferase enzymes
  1. **DNMT1 :** Maintainance methylation
  2. **DNMT 2 :** Maintenance of methylation

  3. **DNMT3a** and **DNMT3b:** **'*de novo*** 'methylases
  4. **DNMT3b**: Methylation of centromeric satellite repeats
  5. **DNMT3L:** Interact with 3a and 3b to stimulate methyl transferase activity

Schematic structure of human DNMTs and DNMT3-like protein. Conserved



. **Conserved methyltransferase motifs in the catalytic domain are indicated in Roman numerals**.

NLS, nuclear localization signal; RFT, replication foci-targeting domain; BAH, bromo-adjacent homology domain;PWWP, a domain containing
a conserved proline-tryptophan-tryptophan-proline motif; PHD, a cysteine-rich region containing an atypical plant homeodomain; aa, amino acids.

- DNMT3L lacks the critical methyltransferase motifs and is catalytically inactive.

Some DNA Methyl Transferases are Essential

Mammalian D*nmts* are essential

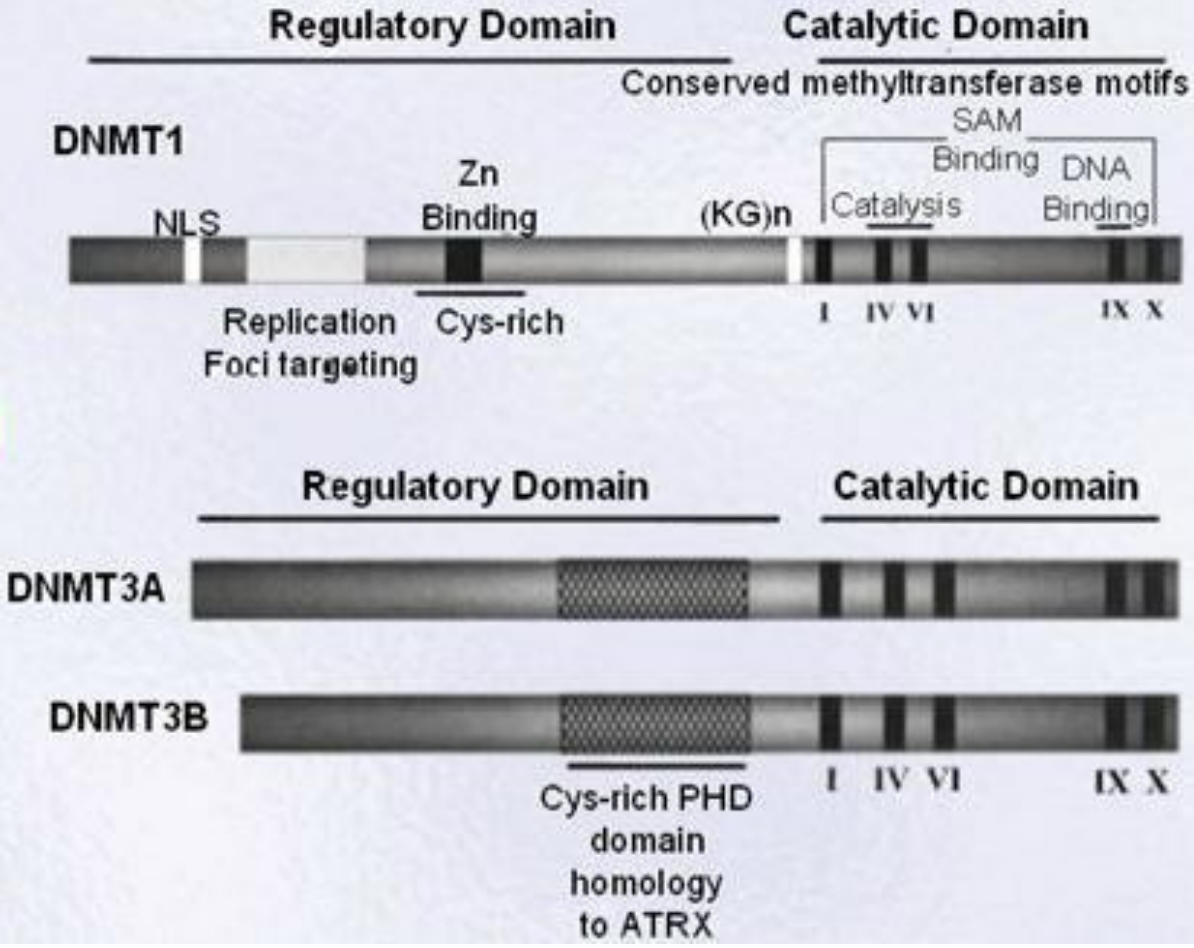
Dnmt1: embryonic lethal

Dnmt2: no obvious effect

Dnmt3a: perinatal death

Dnmt3b: embryonic lethal

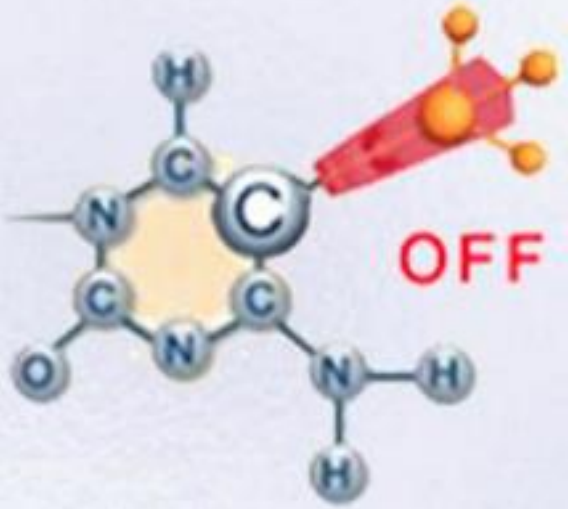
Dnmt3l: no imprints



Some DNA Methyl Transferases are essentials

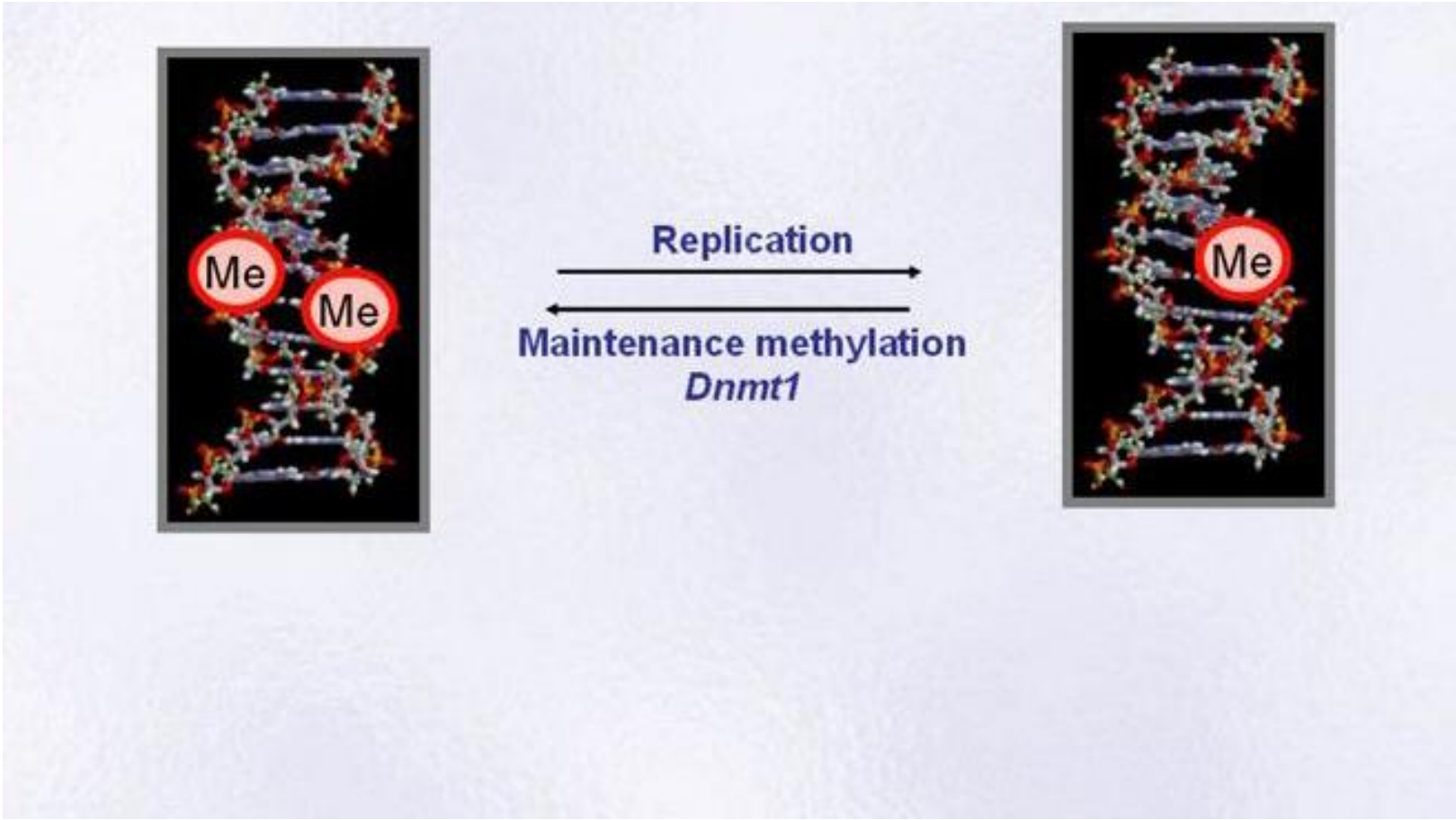
Cytosine Methylation in mammals

- Gene expression
- Chromosomal stability
- Cell differentiation
- Imprinting
- X-Inactivation
- Carcinogenesis
- Aging



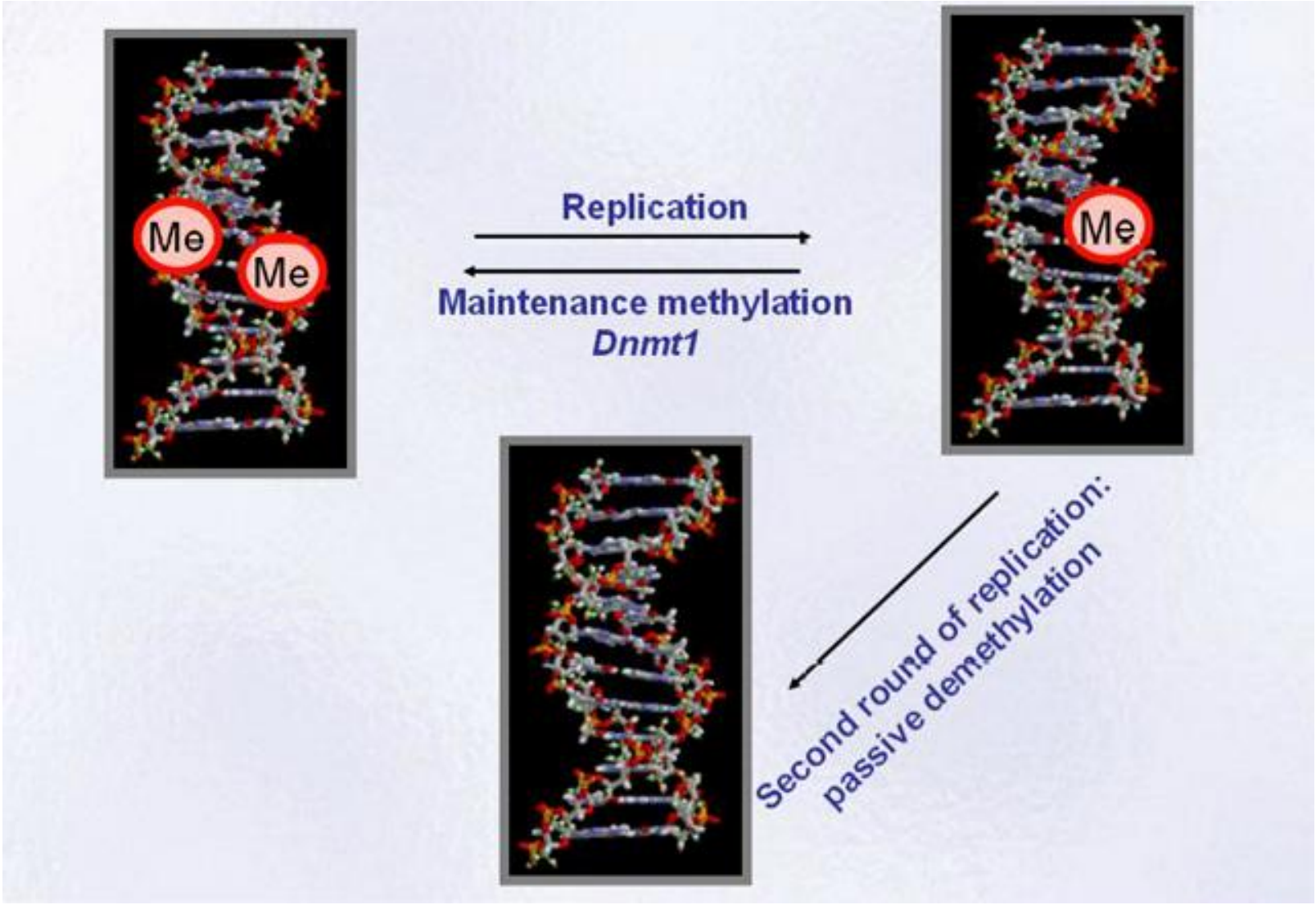
Maintenance of Cytosine Methylation

Establishment and maintenance



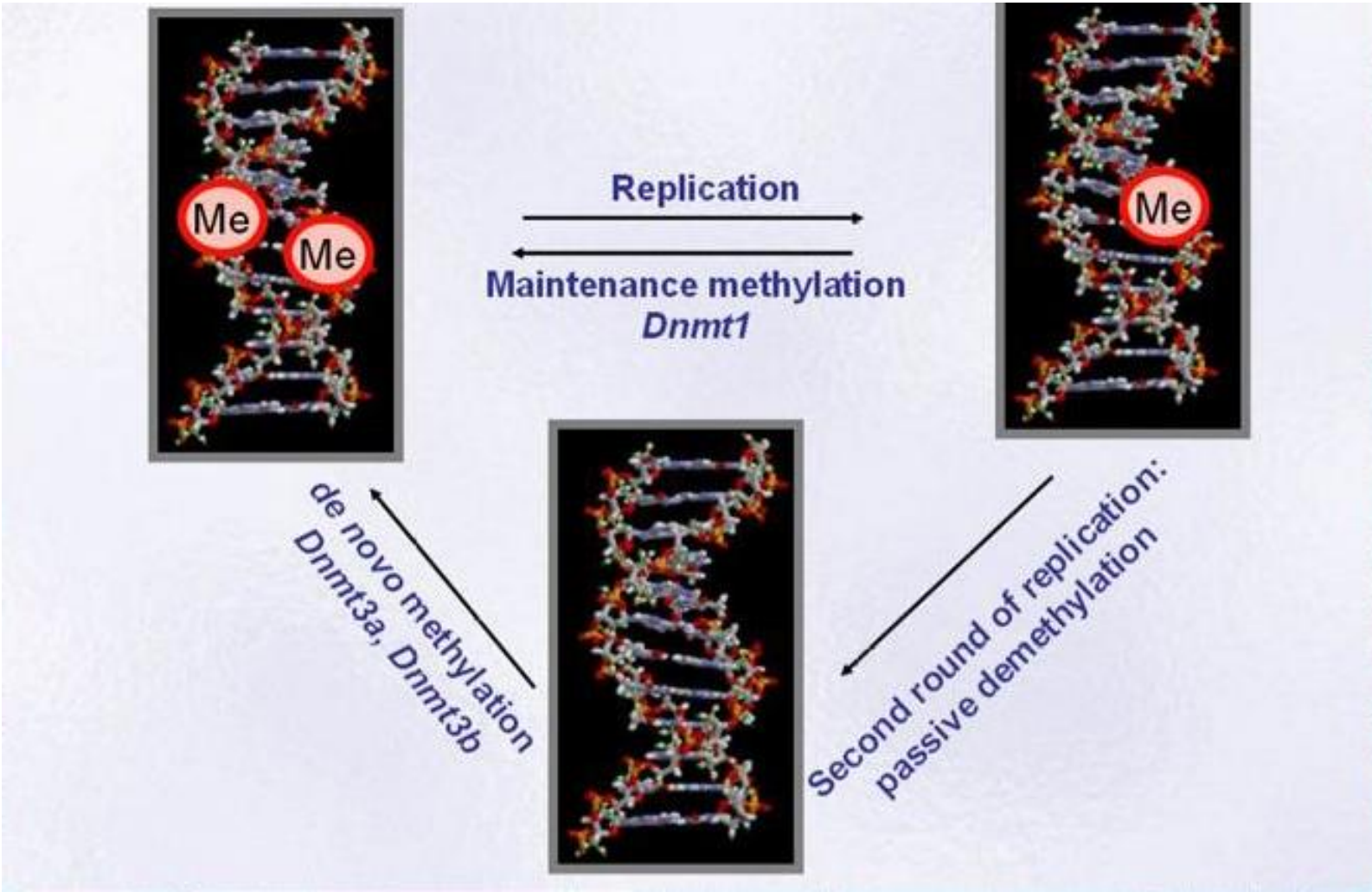
Passive Demethylation of 5-Methyl-Cytosine

Establishment and maintenance

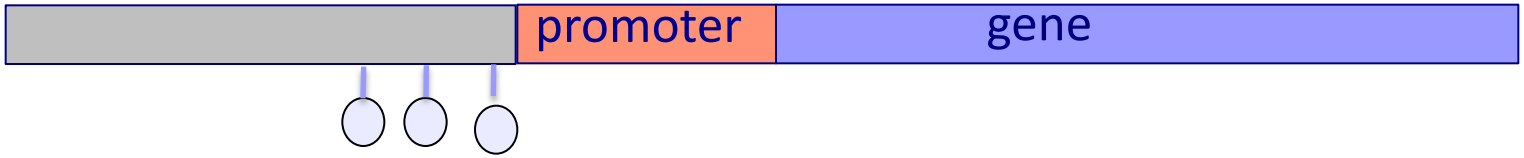


Establishment and Maintenance of Cytosine Methylation

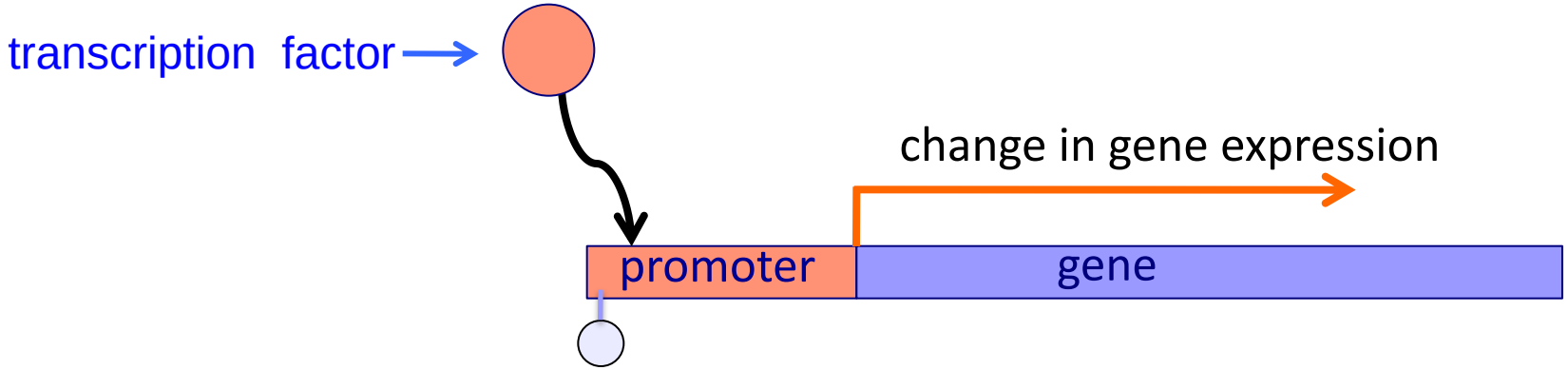
Establishment and maintenance



One way in which DNA Methylation suppresses gene expression

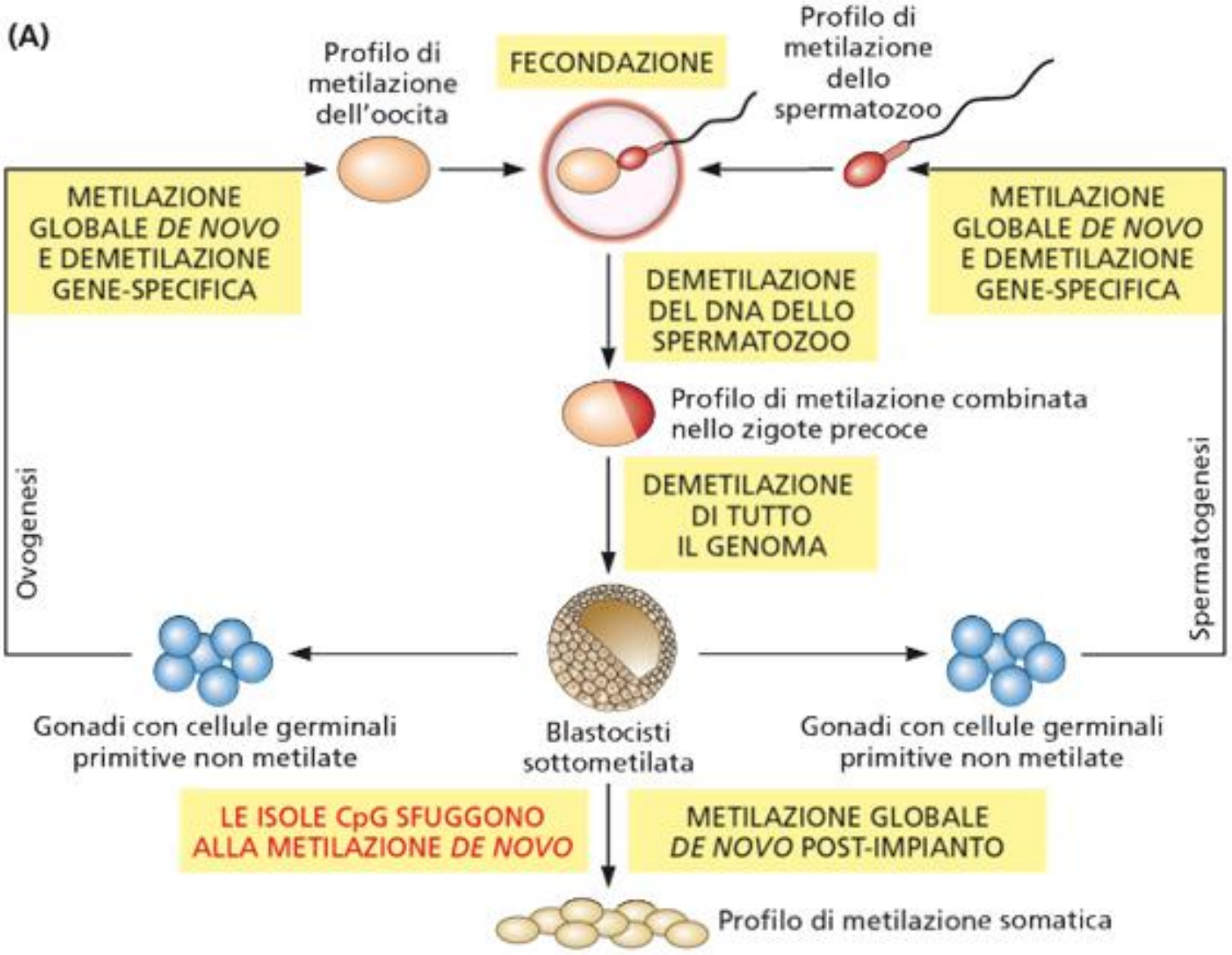


- **Methylation at the promoter** site or upstream of the promoter



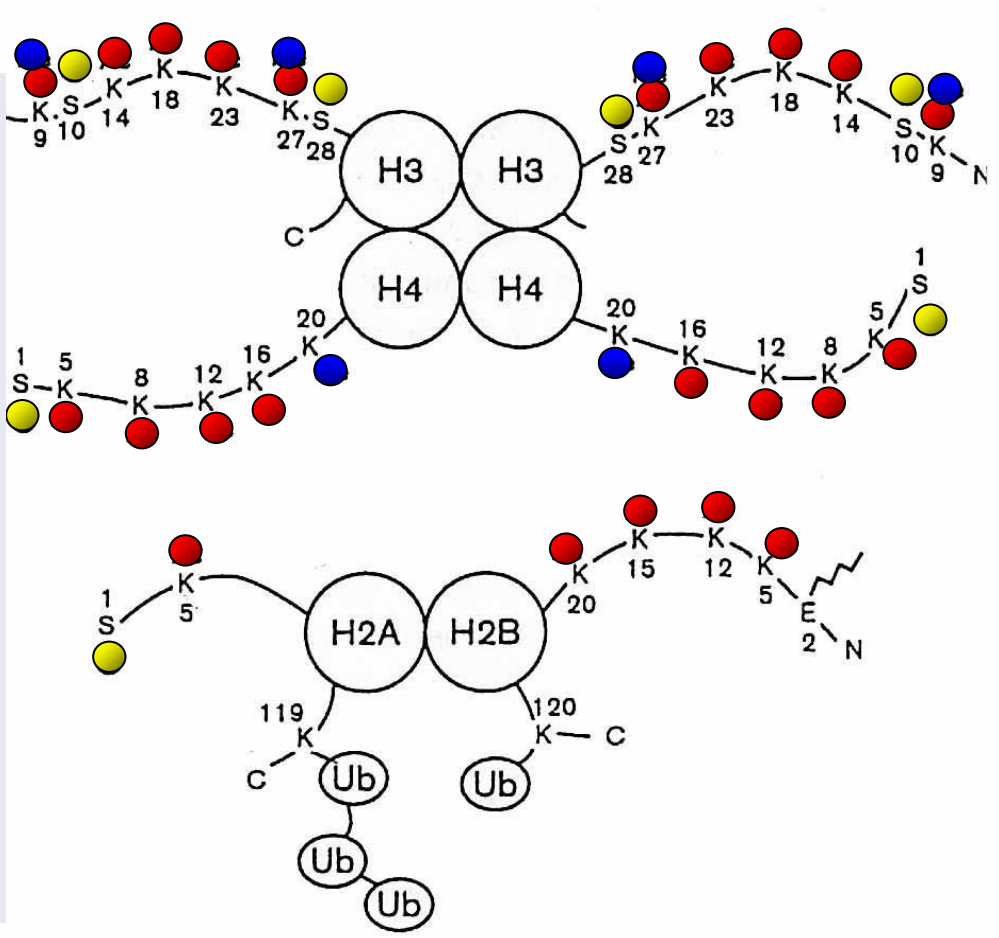
- **Methylation of nucleotides in a regulatory element**, such as the promoter, or upstream of the promoter, **could lead to changes in gene expression.**
- Usually, suppression of expression occurs due to interference with binding of the transcription factor.
- Typically **more methylation** leads to greater suppression of gene expression

La metilazione del DNA è altamente regolata durante lo sviluppo



2a. Histone modifications

- Histones are among the **most conserved** proteins known in evolution,
- but.….
  are also among the **most variable** in post-translational modification.



Methylation

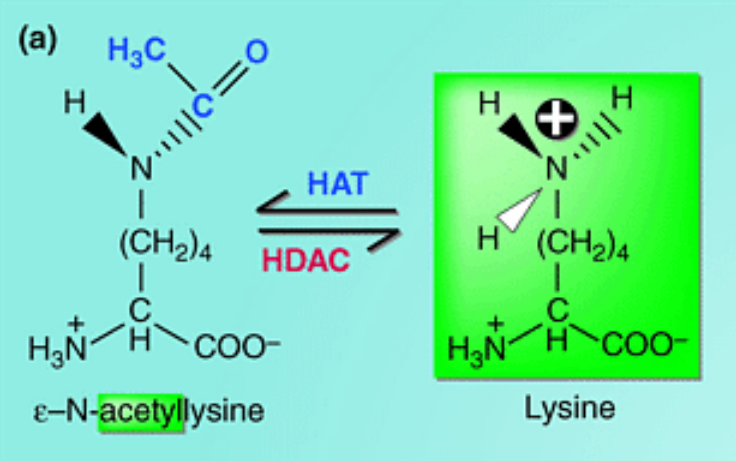
Phosphorylation

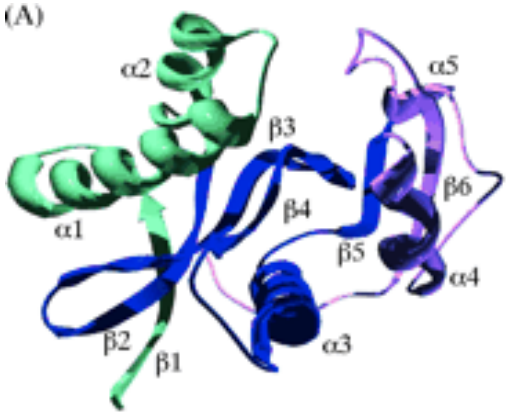
Acetylation

Ubiquitination

ADP-ribosylation

The enzymes: the HATs





yGCN5

- **Histone acetyltransferases (HATs)**
  - Catalyze the transfer of acetyl groups from acetyl-CoA onto histone tails
  - Two main groups: A-type and B-type HATs
    - A-Type HATs catalyze trx-related acetylations
    - B-Type HATs are cytoplasmic and catalyze acetylations linked to transport of newly synthesized histones from the cytoplasm to the nucleus
- **Histone deacetylases (HDACs)**

Functions

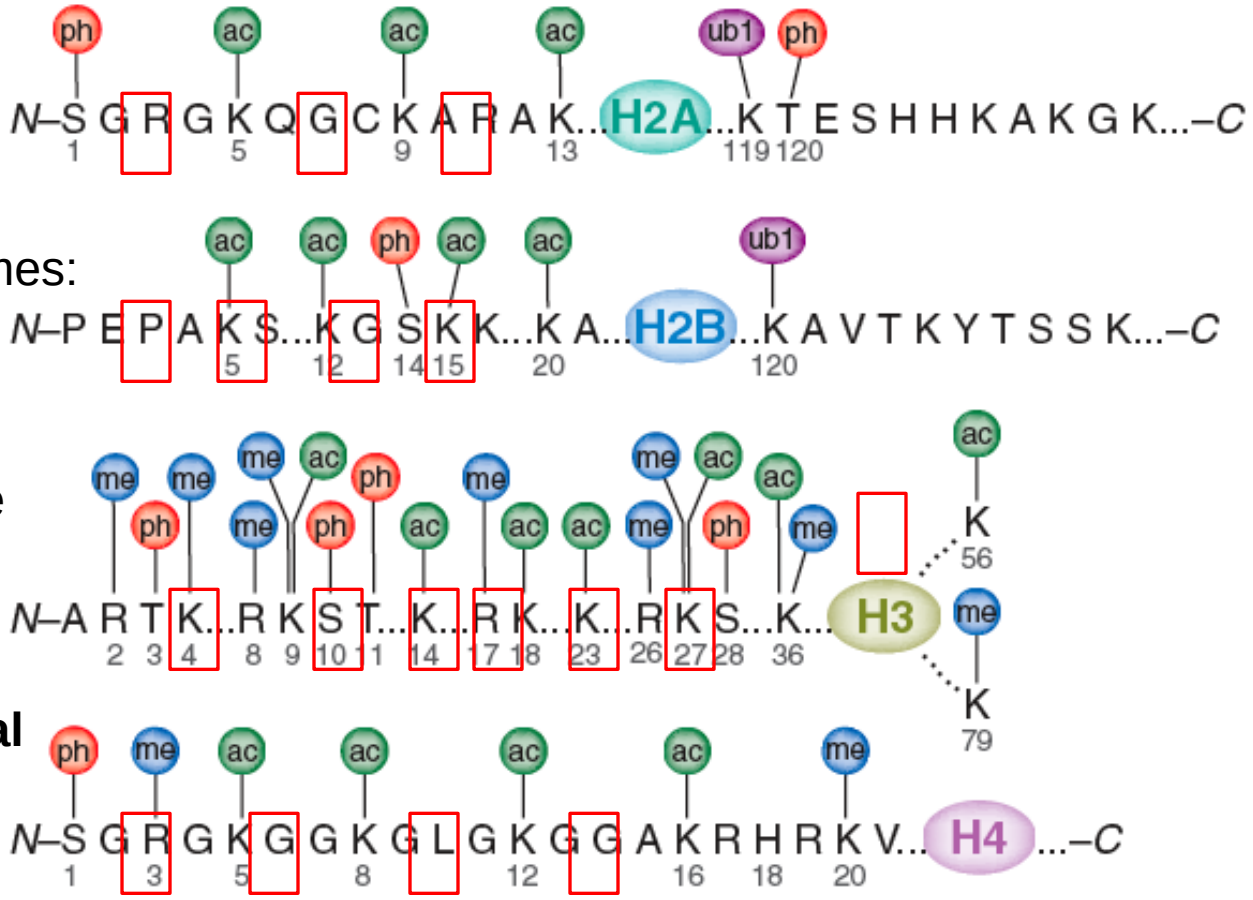
- **Acetylation removes positive charge** of the histone tail, reducing affinity for the negative **charged phosphate groups of** DNA
- It also reduces affinity of tail for adjacent nucleosomes,

  thus affecting ability of nucleosome arrays to form more repressive higher-ordered chromatin structures.
- Also, **acetylation is involved in nucleosome assembly** and interaction of histone with other regulatory proteins, creating a transcription permissive environment.

Lysine Acetylation

➢ **Many lysine residues can be acetylated mainly on histone tails (sometimes in core)**

- Can be part of large acetylation domains



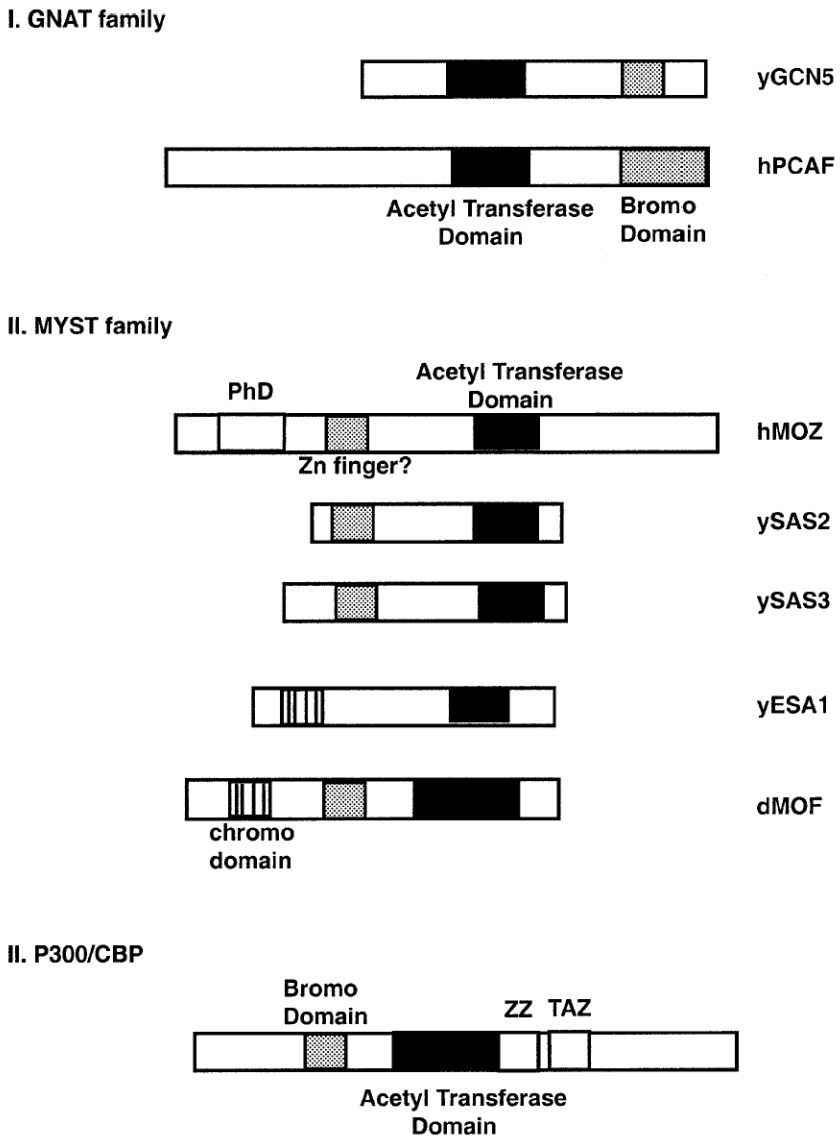
- Modifying enzymes: often **multi-enzyme complexes** can modify multiple residues

- **Well correlated with transcriptional activation**

- Other roles (chromatin assembly, DNA repair, etc.)

HAT families

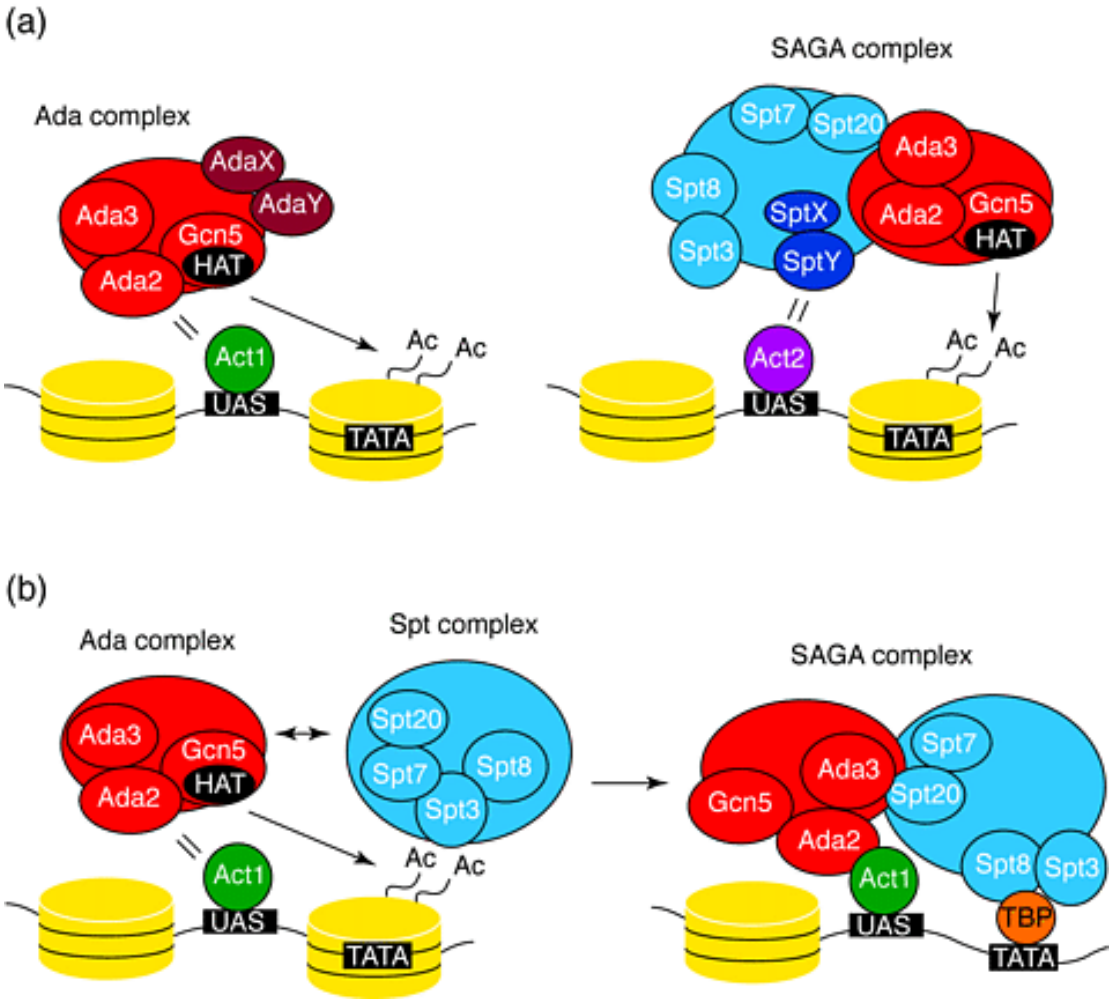
- **GNAT family**
  - Gcn5-related N-acetyltransferase
  - Many contains bromodomains
- **MYST**
  - Named for its founding members: MOZ, Ybf2/Sas3, Sas2, Tip60
  - Several contain chromodomains
- **CBP/p300**
- **GTF-HATs**
  - $TAF_{II}250$
- **Nuclear receptor linked HATs**
  - SRC1, ACTR



HATs and coactivators in the same complex

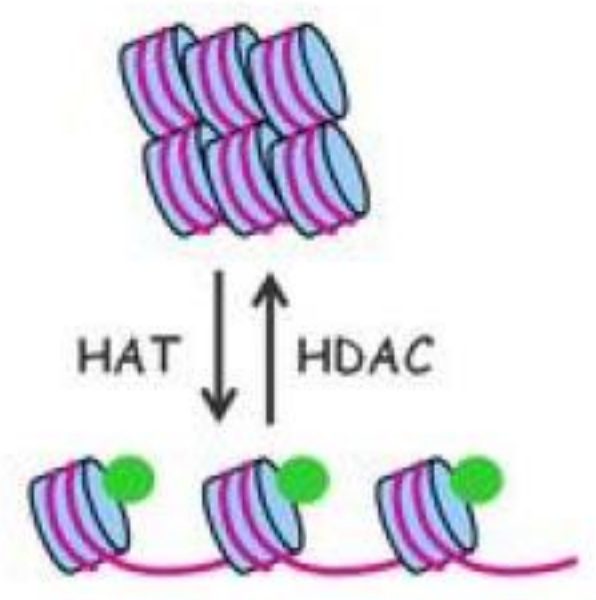
HAT: local acetylation

coactivator: bridge



Deacetylases

- Multi-enzyme complexes
- Targeted by transcriptional repressors
- Deactylate histone tails



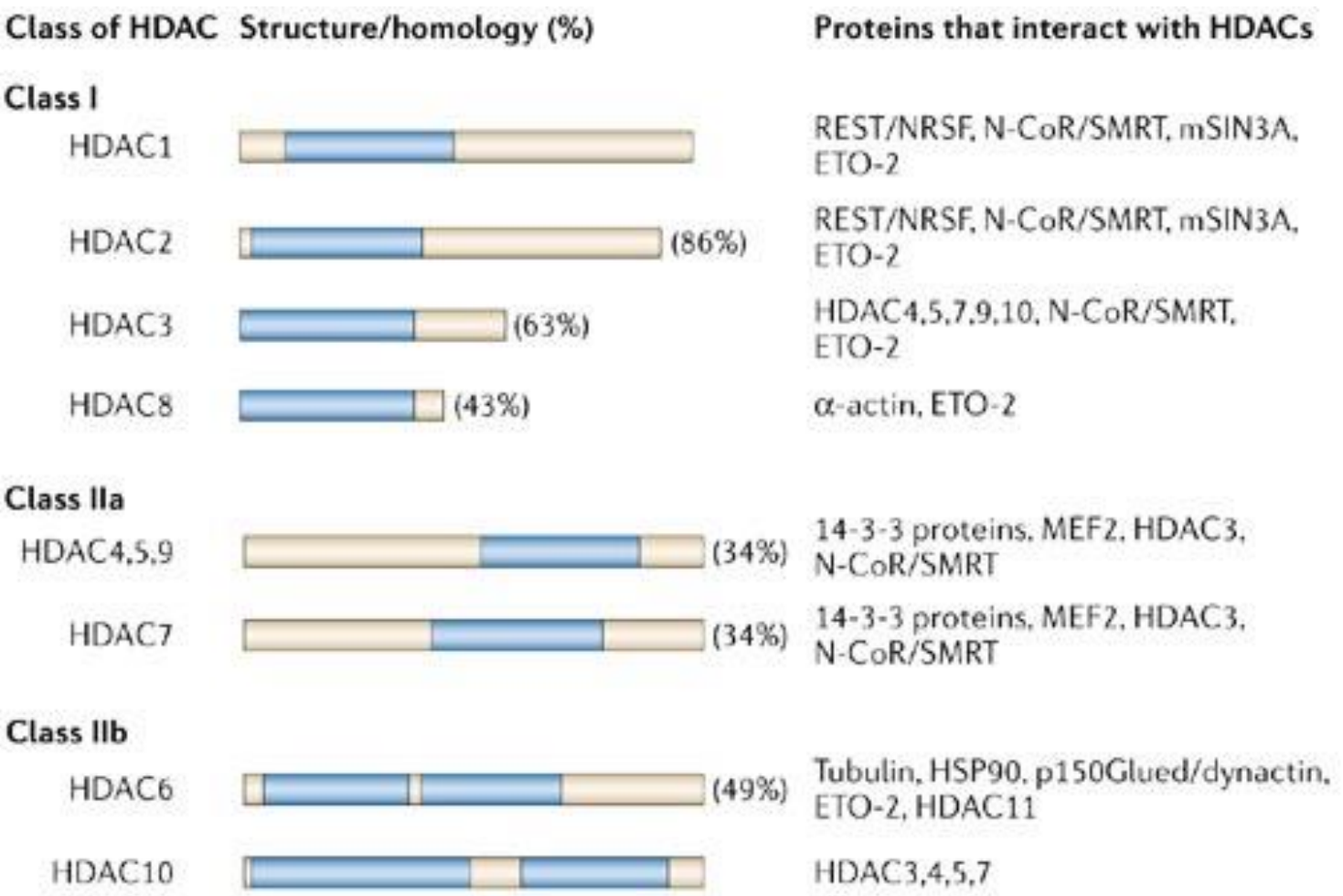
HDAC Superfamilies



- **Class I HDACs**
  - RPD3-like (HDAC 1, 2, 3)
  - most cell types
  - in nucleus
- **Class II HDACs**
  - HDA1- like (HDAC 4, 5, 6, 7, 9, 10)
  - HDAC and N-term repressor motif
  - restricted expression
  - shuttle in/out nucleus
- **Class III HDACs**
  - Sir-2 (NAD-dependent)
  - sirtuins



HDACs are in Complexes



Butler and Bates, *Nature Reviews Neuroscience,* 2006.

Cartoon schematic illustrating the Rpd3L, Rpd3S, and Rpd3 complexes.

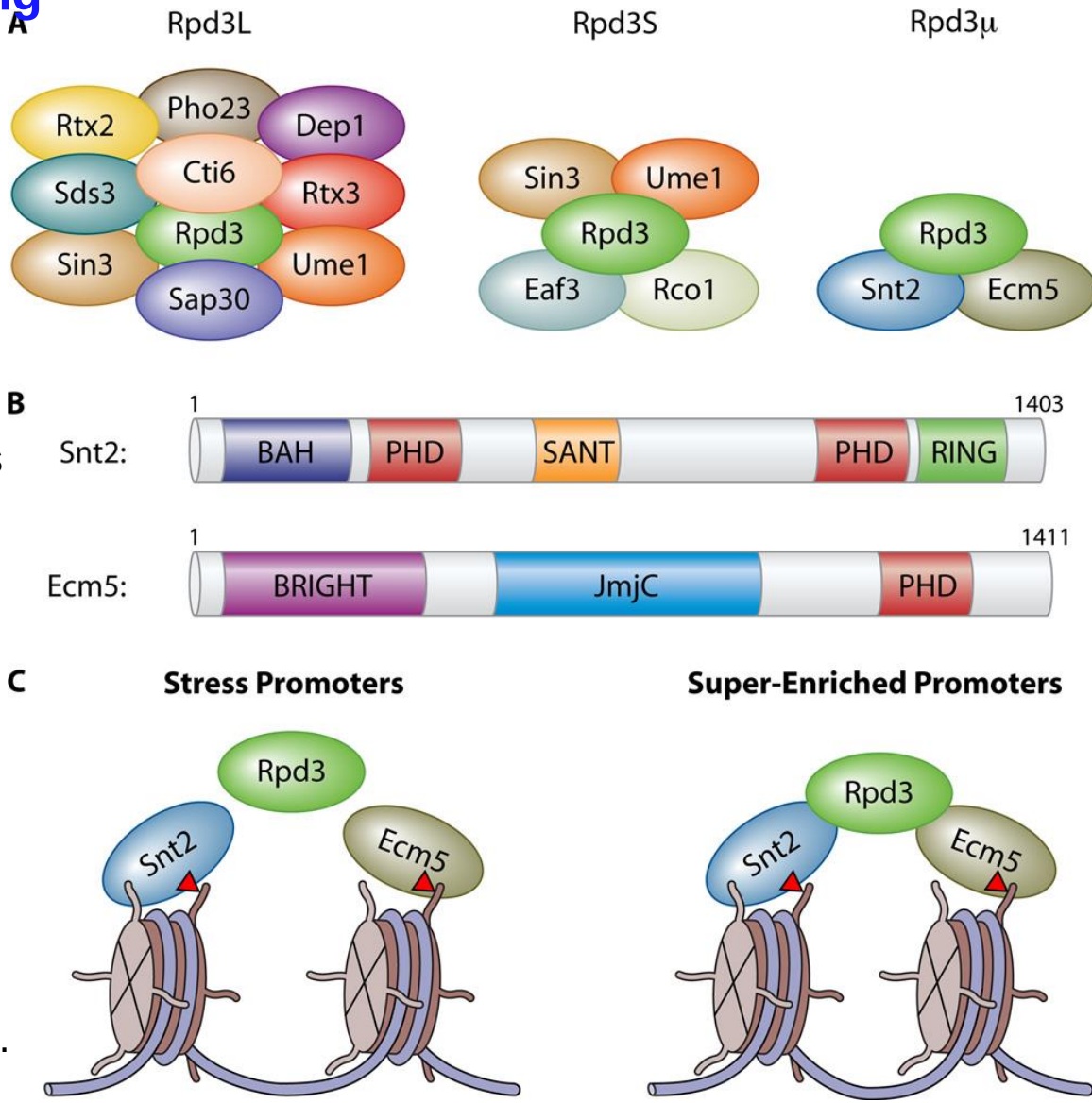
(B) The domain architecture of Snt2 and Ecm5 reveals multiple, putative chromatin and DNA binding domains.

(C) Rpd3 localizes to two distinct types of promoters.

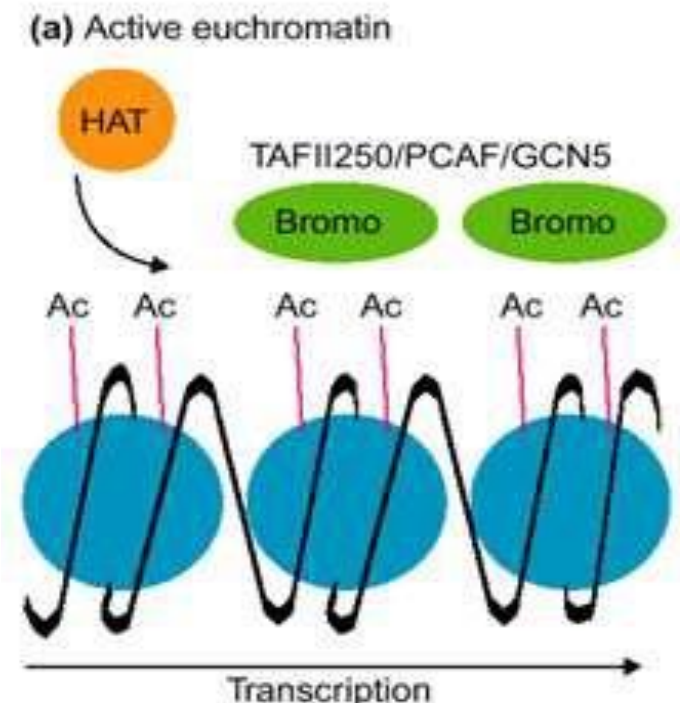
- Upon oxidative stress, all 3 members of theRpd3 complex are recruited to oxidative stress response promoters, though Rpd3 recruitment is not dependent on Snt2 or Ecm5.

The superenriched promoters , and recruitment of Rpd3 is dependent upon both Snt2 and Ecm5.

Recent work suggests that Ecm5 may bind histone H3 lysine 36 trimethylation (red triangles).

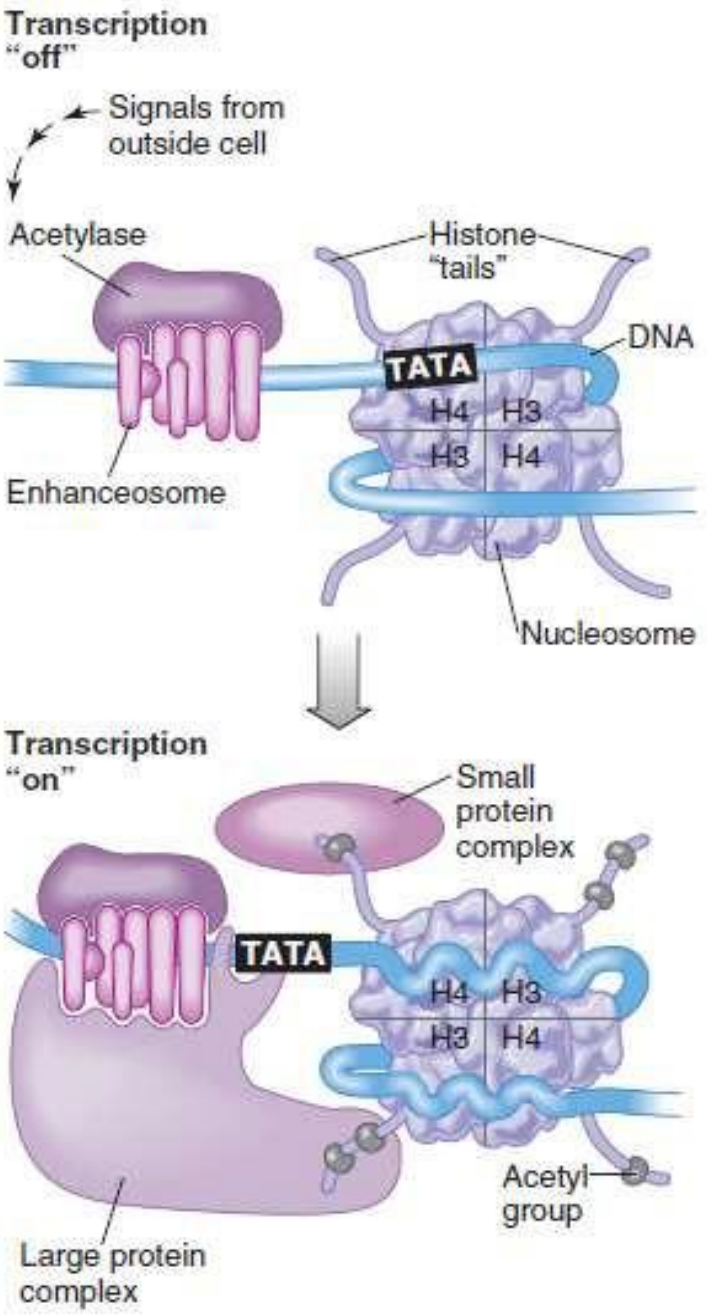


- **Modification of histone tails** by **acetylation is known to increase the access of transcription factors** to DNA through structural changes in nucleosomes or nucleosomal arrays.
- **Acetylated histones are also specifically recognized by other proteins**.
- The **bromodomain**, found in transcription factors and HATs **allows for the preferential recognition of histone tails** when they are **acetylated at specific lysine residues**



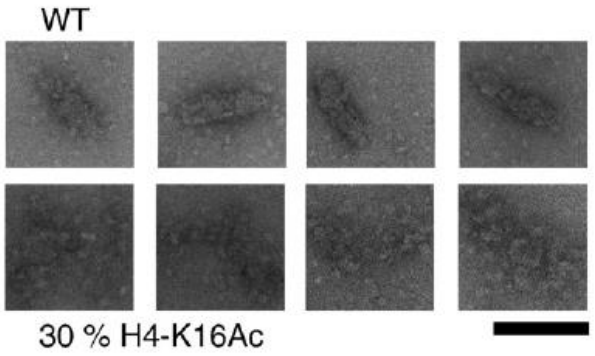
Acetylated histones allow transcription to begin.

- Once the **acetyl groups are added** to **particular amino acids in the tails** of certain histones,
- the **TATA box becomes accessible to transcription factors**.



Roles of Acetylation

1. **Opens up chromatin:**
   - Reduces charge interactions of histones with DNA (K has a positive charge)
   - Prevents chromatin compaction (H4K1 prevents 30nm fiber formation)

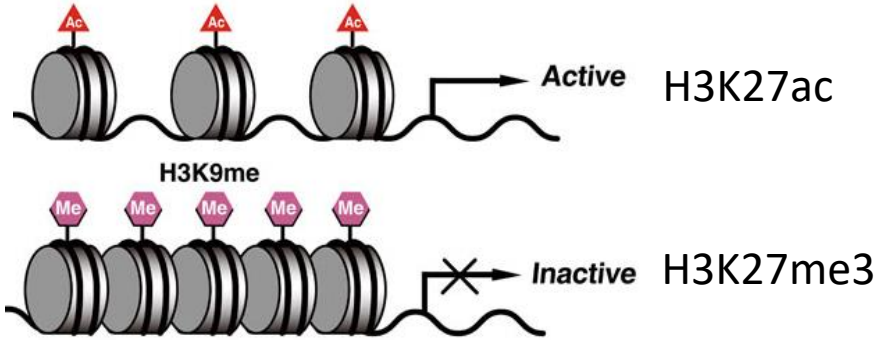


Robinson et al., J. Mol. Biol., 2008.

2. **Recruits chromatin proteins with bromodomains**

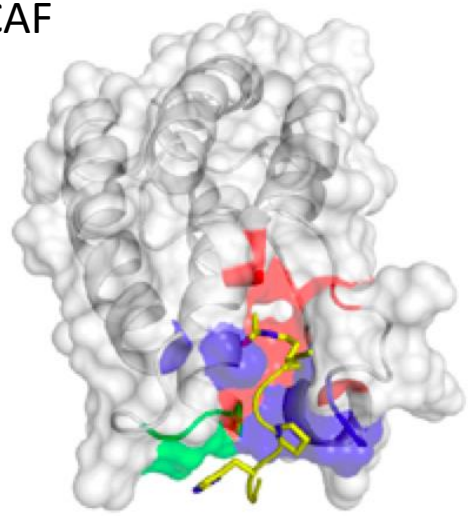
   (SWI/SNF, HATs: GCN5, p300)

3. May occur at same residues as methylation repressive effect (**competitive antagonism**)



Yang and Chen, Cell Research, 2011.

PCAF

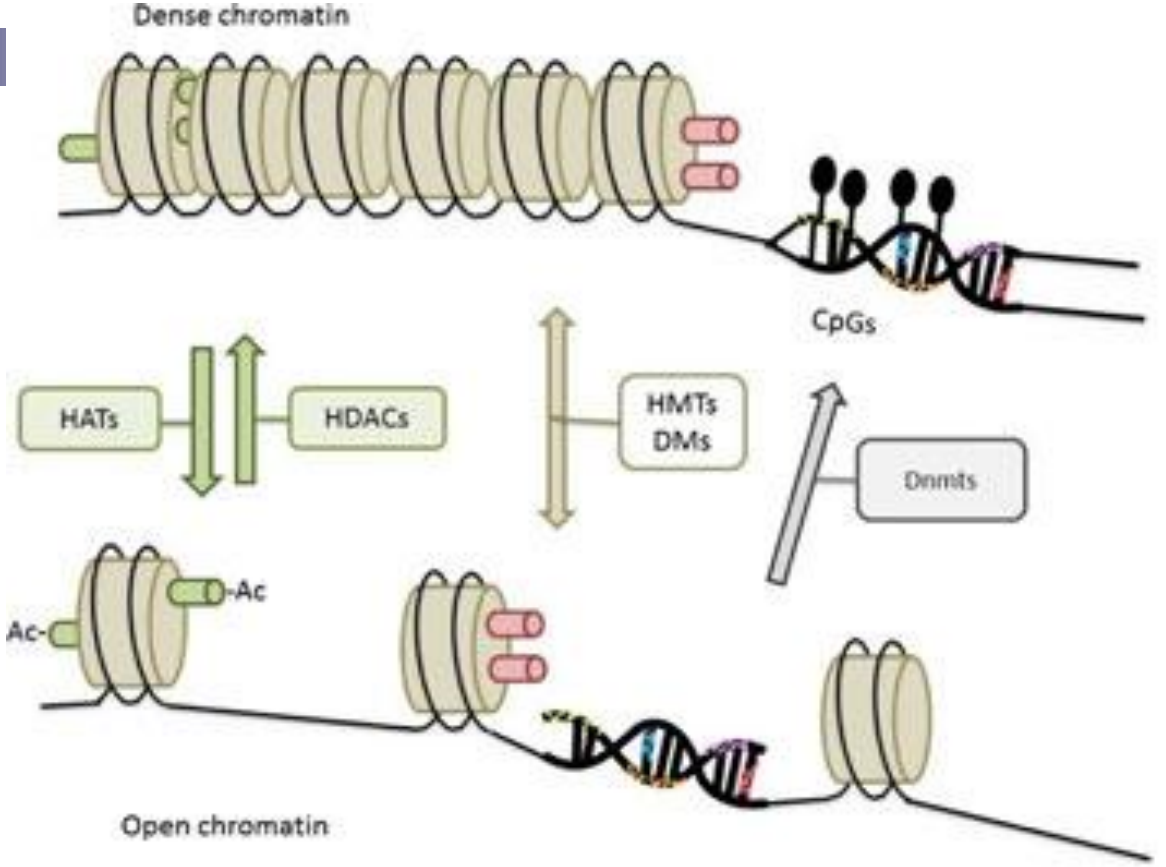


Mujtaba et al., Oncogene, 2007.

Chromatin regulation mediated by histone modifications.

Histone acetyltransferases (HATs), histone deacetyl-Transferases (HDACs), histone methyltransferases (HMTs), demethylases (DMs) **Are enzymes involved in forming open or dense chromatin**.

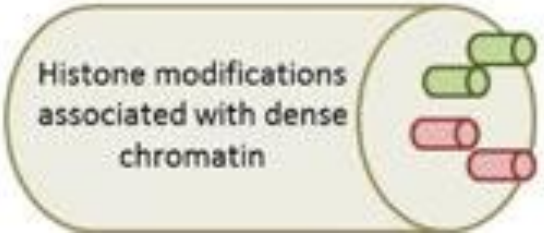
(b) Some key histone modifications associated with open or dense chromatin states. Model created with data from Verona et al. (61).





H3ac (acetyated H3-lysin)
H4ac (acetyated H4-lysin)

H3K4me2 (di-methylated H3-lysin 4)
H3K4me3 (tri-methylated H3-lysin 4)



Deacetylated histones

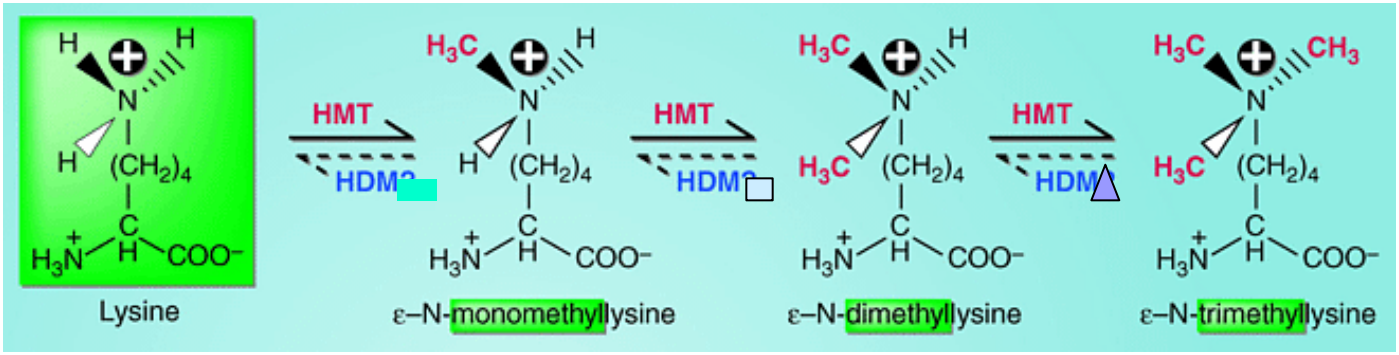
H3K9me3 (tri-methylated H3-lysin 9)

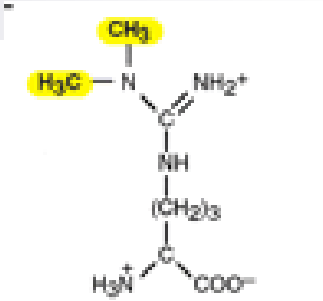
2b. Histone methylation

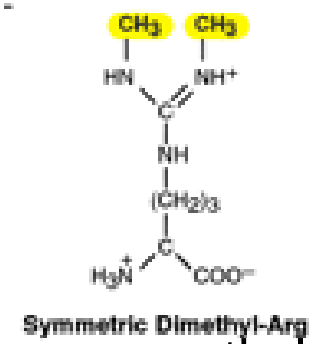
A role in both activation and repression

Histone methylation

multiple variants on Lys and Arg







- **Both Lys (K) and Arg (R) can be methylated** at more than one methyl-group
- Histone methylation is a relatively stable modification with a slow turnover rate.
- There are two classes of enzymes involved in histone methylation: **methyltransferases and demethylases.**
- While methyltransferases are responsible for establishing methylation patterns, **demethylases** are capable of removing methyl groups not only from histones but other proteins as well
- An ideal epigenetic mark for more long-term maintenance of chromatin states. Methylated residues are present both in eu- and hetero-chromatin.

The enzymes:

Histone lysine methyl-transferases (HKMTs)

- The first HKMT, SUV39H1
  - was identified in 2000.
  - It specifically **methylates Lys 9 of histone H3.**
  - Later several other HMTs have been identified (see table below)
- A **SET-domain** is required for HKMT activity
- 5 lysines within H3 (K4, K9, K27, K36 and K79) and 1 lysine within H4 (K20) are methylated by specific HKMTs

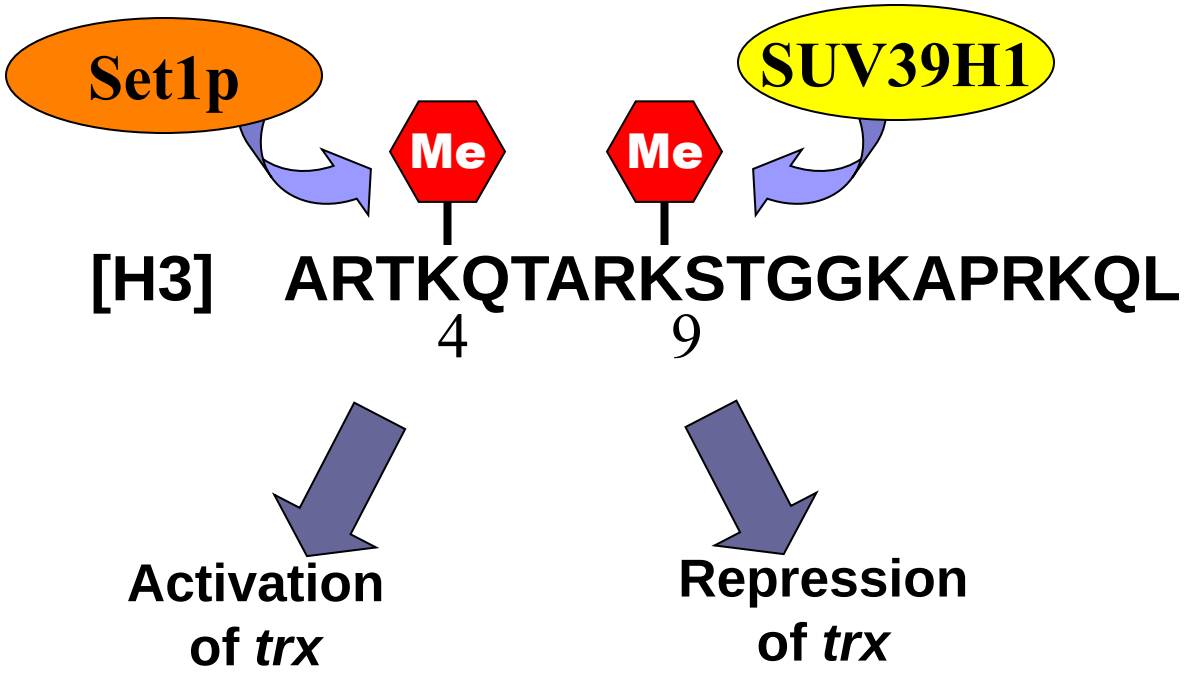
Table 1. Histone lysine (K) methyltransferases (HKMTs)[a]

	Enzymes (catalytic subunits)			
Site specificity[b]	***S. cerevisiae***	***S. pombe***	***D. melanogaster***	**Mammals**
H3-K4	Set1 [89–91]	Set1 [92]	Trx, Ash1[c] [37]	Set9[d], ALL-1, MLL, ALR-1 and 2 [93], ALR [93], Set1 [68]
H3-K9	Absent	Clr4	Su(var)3–9 [10], Ash1[c] [37]	Suv39 h, G9a, Eu-HMTase I [11], ESET[e], SETBD1
H3-K27	Absent	?	E(Z)	Ezh2, G9a
H3-K36	Set2 [94]	?	?	NSD1 [67]
H3-K79	Dot1	?	?	Dot1L [60]
H4-K20	Absent	?	PR-Set7,Ash1[c] [37]	PR-Set7

Function - Activation or repression?

2 sites - 2 HKMTs - 2 effects

- **Histone methylation was traditionally linked to repression**, but turns out to be linked also to activation
  - H3 K9 methylation correlates with heterochromatin formation.
  - Met-K4 in H3 correlates with *trx* activation
  - Set1p associated with HAT complex

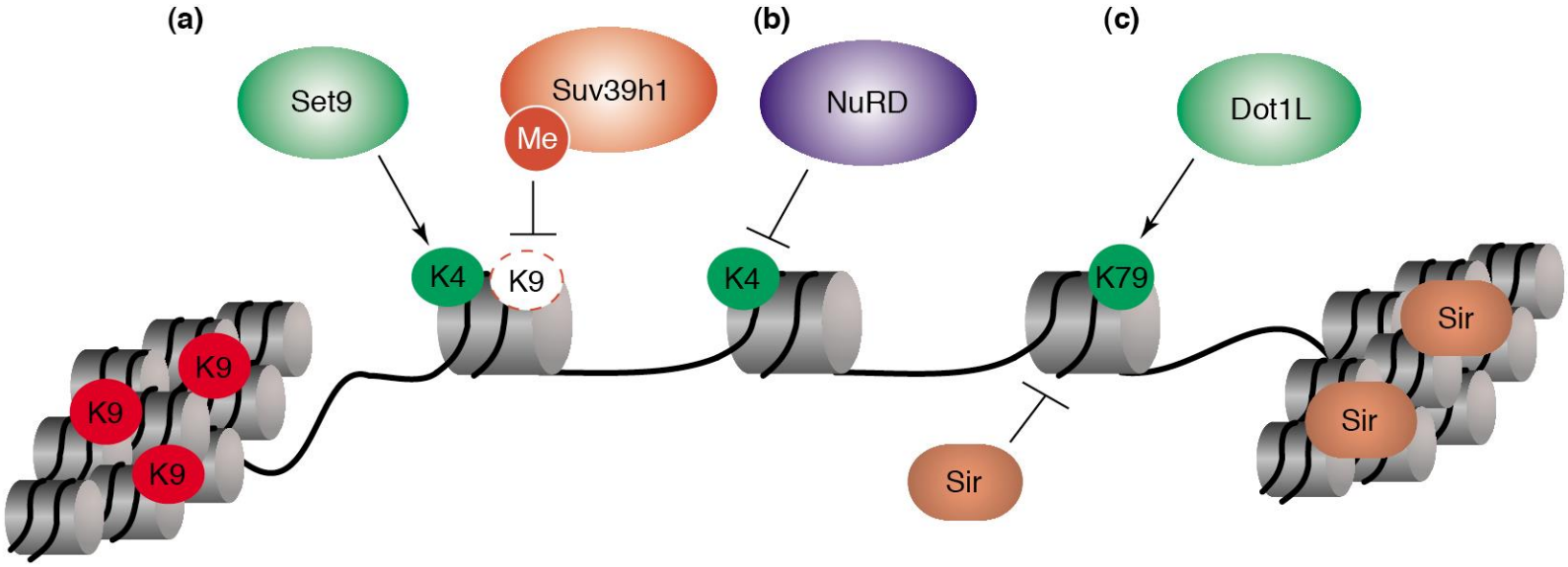


Function of histone methylation

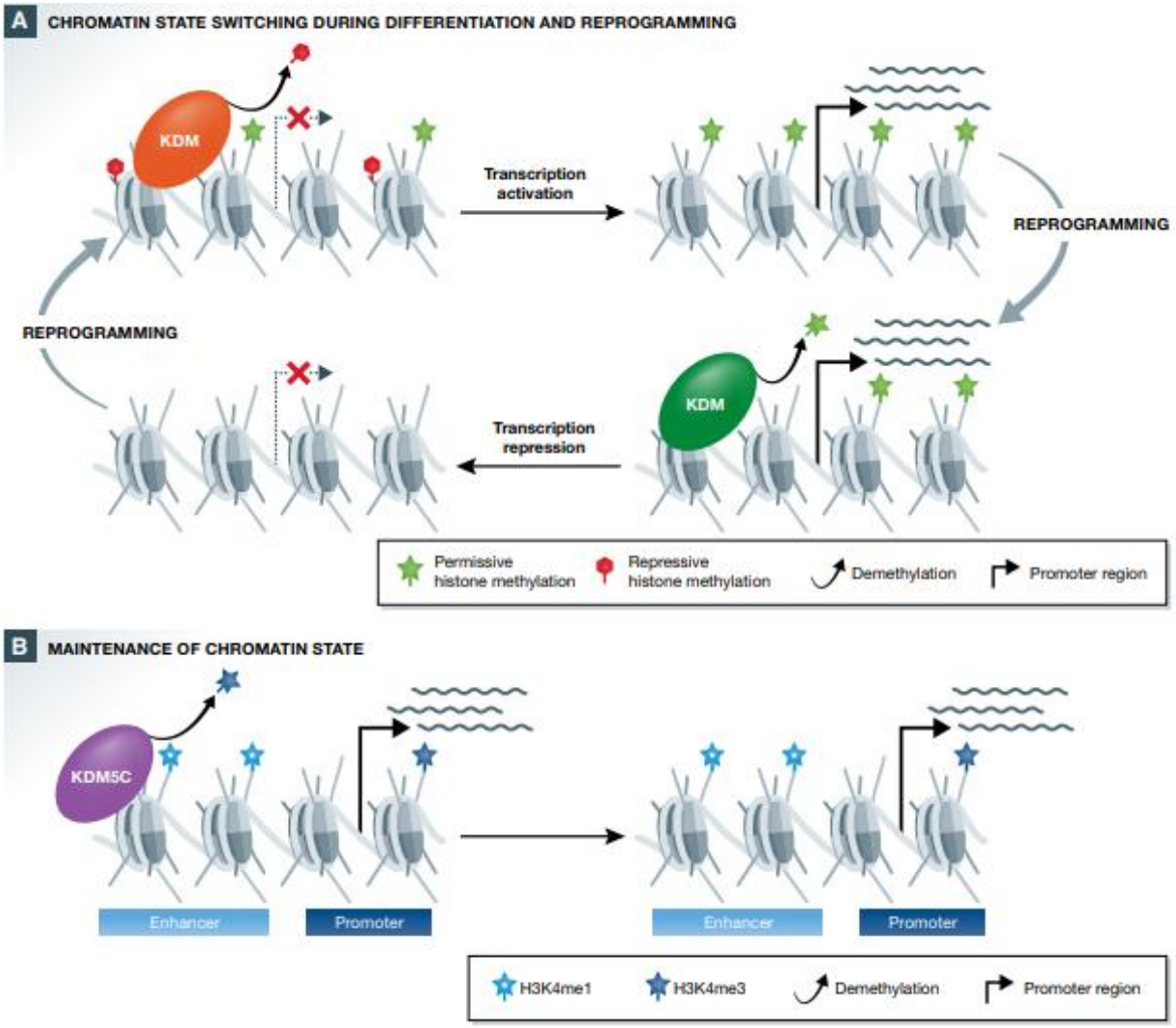
- **Repression** (H3-K9, H3-K27, H4-K20) - more next lecture
  - Numerous reports on silencing effects linked to H3-K9 methylation
  - Heterochromatin formation linked to H3-K9 methylation and HP1 binding
  - Highly condensed centromeric regions related to methyl addition to H3-K9.
  - H3-K9 and H3-K27 methylation might be important for the X chromosome inactivation process.
- **Activation** (**H3-K4**, H3-K36, H3-K79)
  - H3-K4 methylation is generally associated with trx active chromatin.
  - di-methyl H3-K4 appears to be a global epigenetic mark in euchromatic regions and tri-methylation of H3-K4 correlates with active transcription
  - both H3-K4 and H3-K79 participate in establishing euchromatic regions by preventing the spreading of heterochromatic regions.
- Role of histone lysine methylation in trx elongation

Activating effects of methylation - two levels

- **Chromosome level - preventing spread of heterochromatin**
  - The methylation of H3-K4 specifically impairs methylation at H3-K9, thereby blocking a major pathway of heterochromatin formation
  - binding of the histone deacetylase NuRD repression complex to the H3 N-terminal tail is precluded by methylation at K4, but not K9
- **Gene level**
  - di-methyl H3-K4 appears to be a global epigenetic mark in euchromatic regions and tri-methylation of H3-K4 correlates with active transcription
  - Elongation - more later



Histone demethylases shape chromatin architecture at gene regulatory elements to regulate gene expression.



- A) Demethylases actively remove histone methylation to establish new chromatin environments at gene regulatory elements.

Removal of repressive modifications, such as

- H3K27me2/3, helps to create t transcriptionally

permissive modifications, such as H3K4me3, contributes to the formation of more repressive chro[illegible] important in achieving new gene expression programs during lineage commitment and cellular reprogramming. B) Histone demethylases play a key role in the maintenance of established chromatin states by preventing the spurious accumulation of alternative histone methylation states. For example, the H3K4me2/3 demethylase KDM5C contributes to the maintenance of enhancer identity by maintaining local H3K4me1 levels

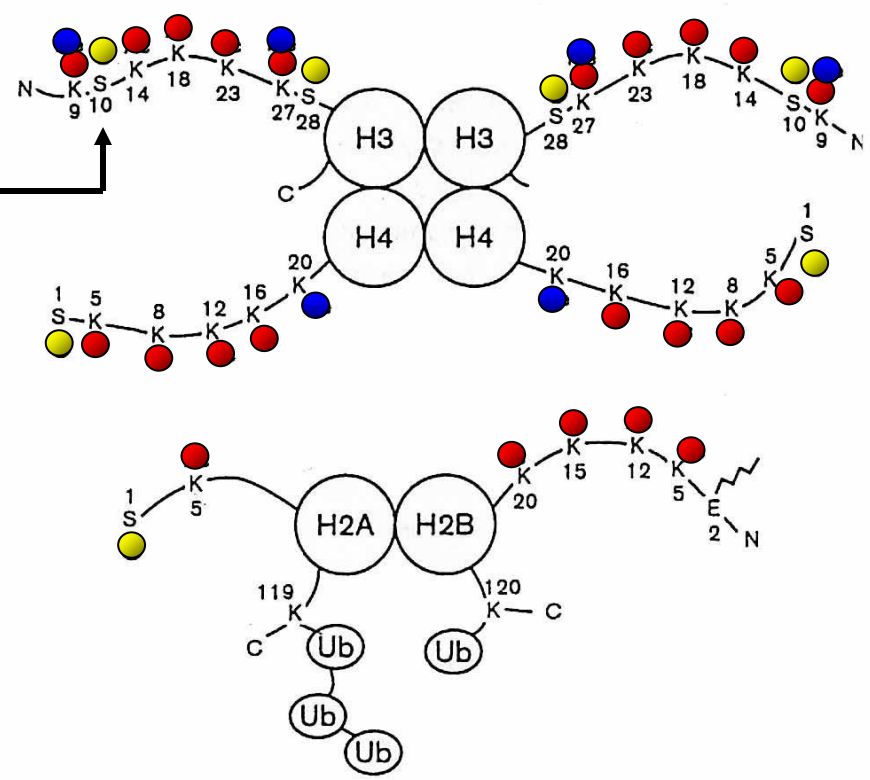
Other histone modifications

Other histone modifications

Phosphorylation

H3 Ser10 - mitosis associated
EGF-stimul - Rsk2 mediated phosphorylation of S10 in H3
Phospho-S10 may enhance acetylation of surrounding residues

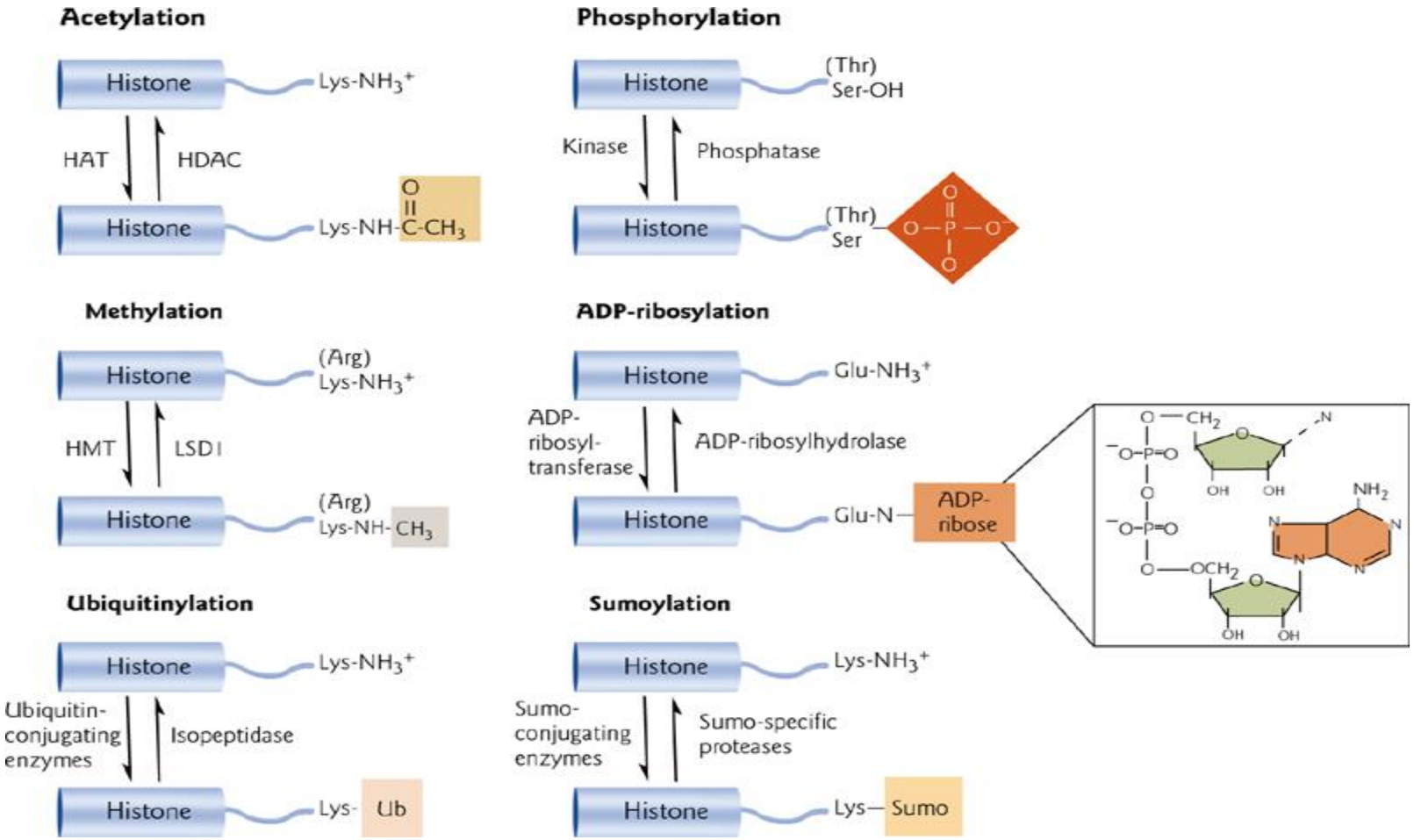
Methylation



Methylation
Phosphorylation
Acetylation

Ubiquitination
ADP-ribosylation

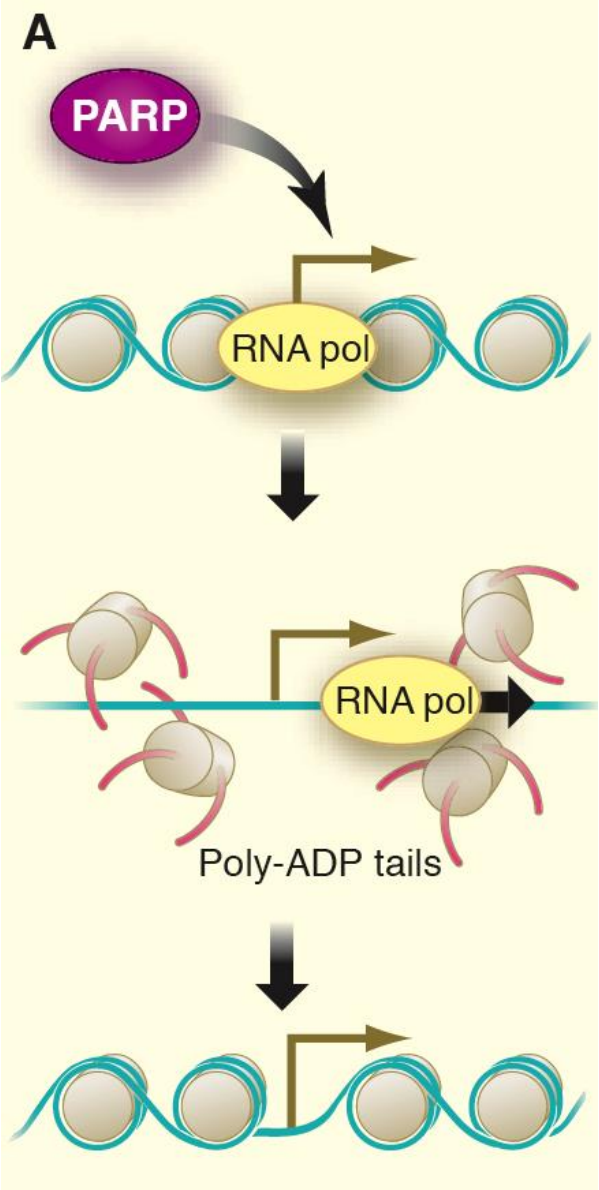
Post-translational Modification of Histone N-terminal Tails



Wide Range of Histone Modifications to Regulate the "STATE" of Chromatin
Proteins with bromodomains (acetate groups) or chromodomains (CH_3)

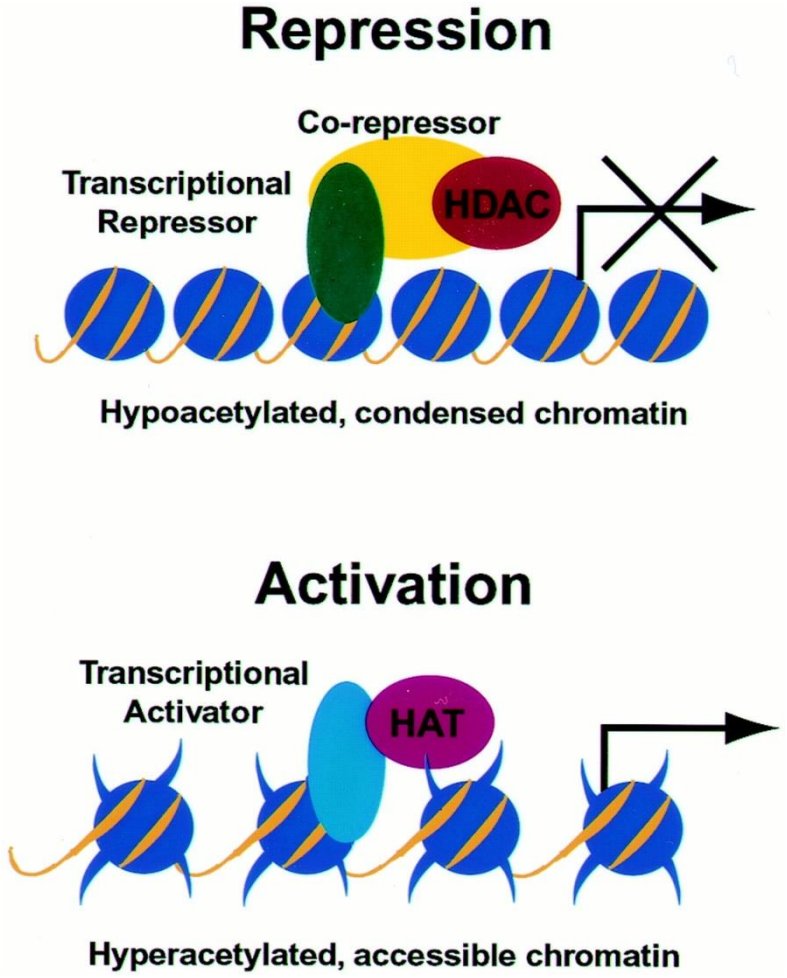
Poly-ADP-ribosylation (PARP)

- **PARP recruitment**
  - During activation, PARP is recruited by TFs to condensed chromatin (green).
- **Modification = Poly-ADP-ribosylation**
  - PARP induces dissociation of nucleosomes (beige) by adding long ADP-ribose tails to their histone proteins.
- **Stays open**
  - PARP prevents rebinding of transcription factors to the DNA by adding ADP-ribose tails to them

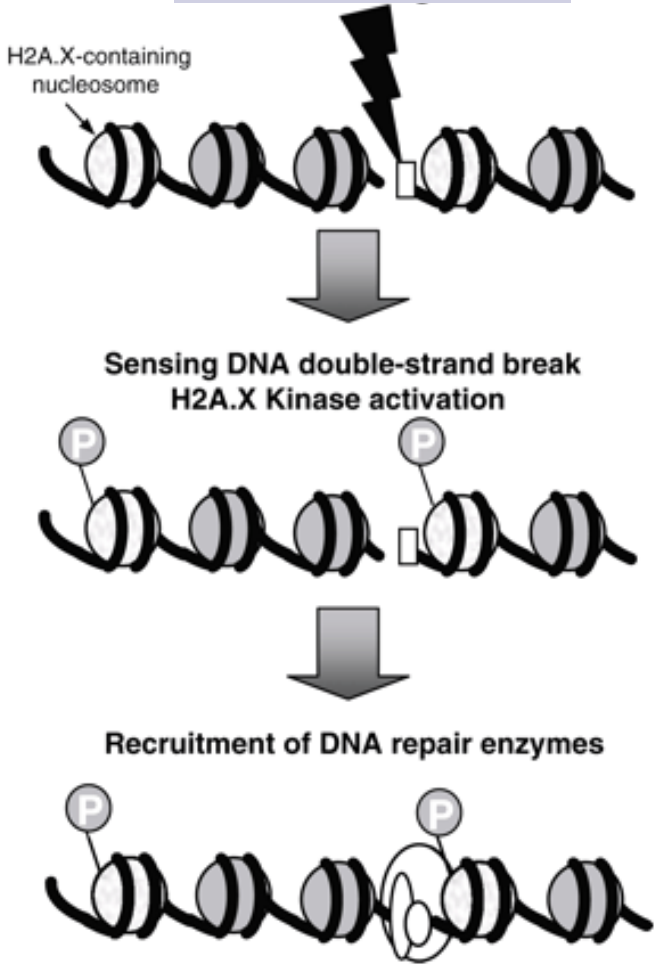


General Roles of Histone Modifications

Gene Regulation



DNA Damage



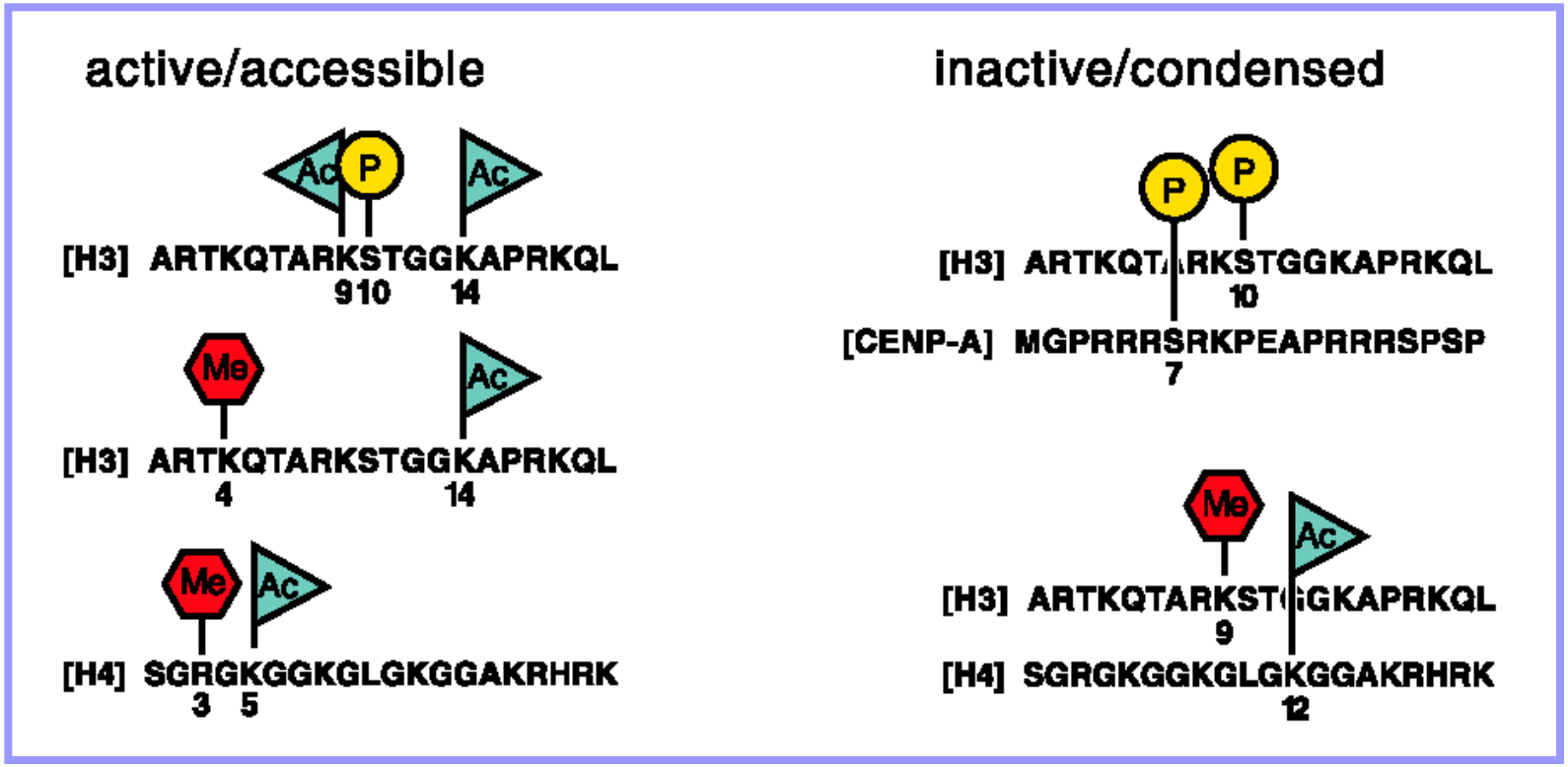
Moggs and Orphanides, Toxicological Sciences, 2004.

Combinatorial possibilities large - a language?

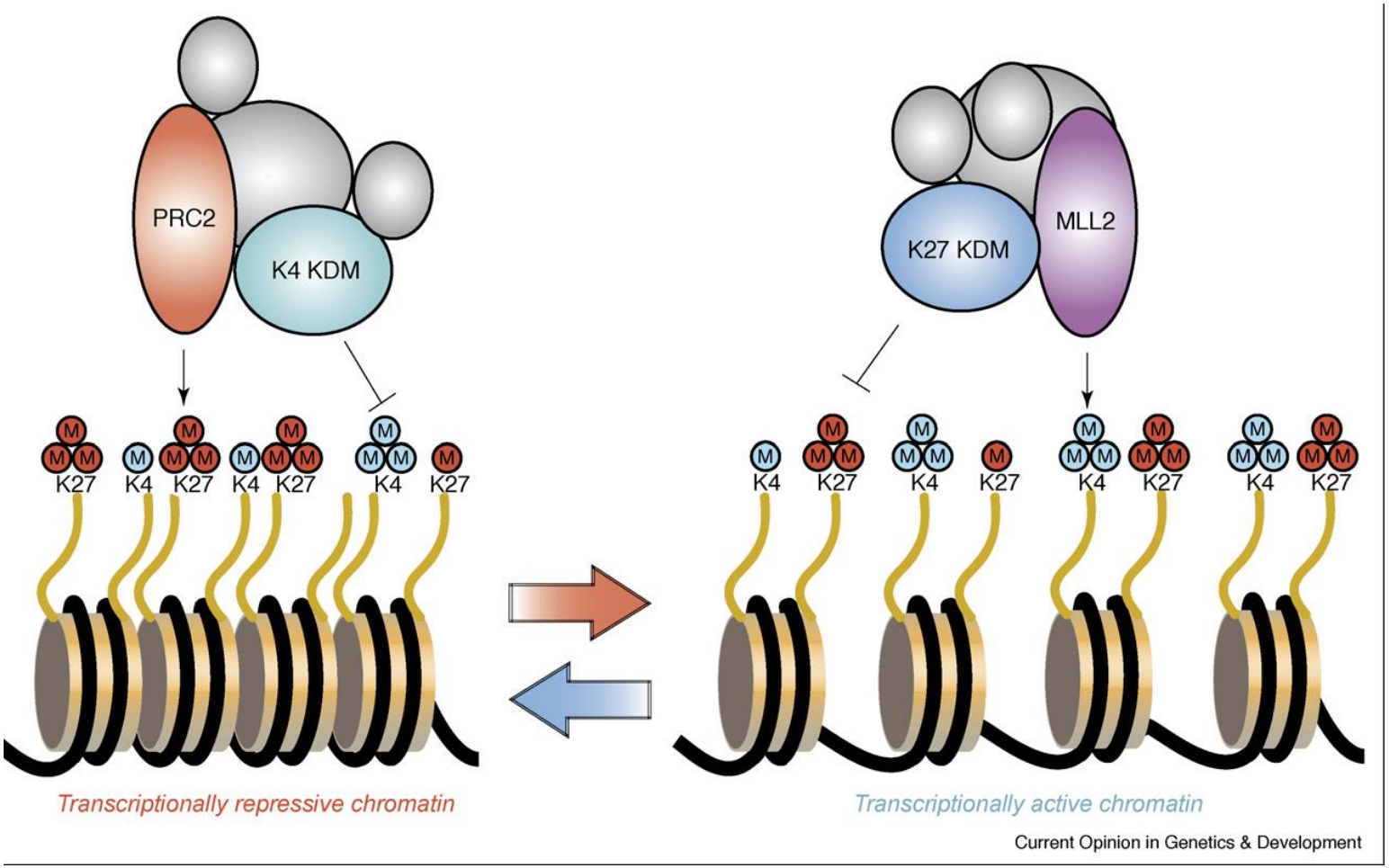
- **Acetylated = active**, **deacetylated = inactive**

  True but probably too simple

- **Many combinations** - link to active/inactive chromatin complex



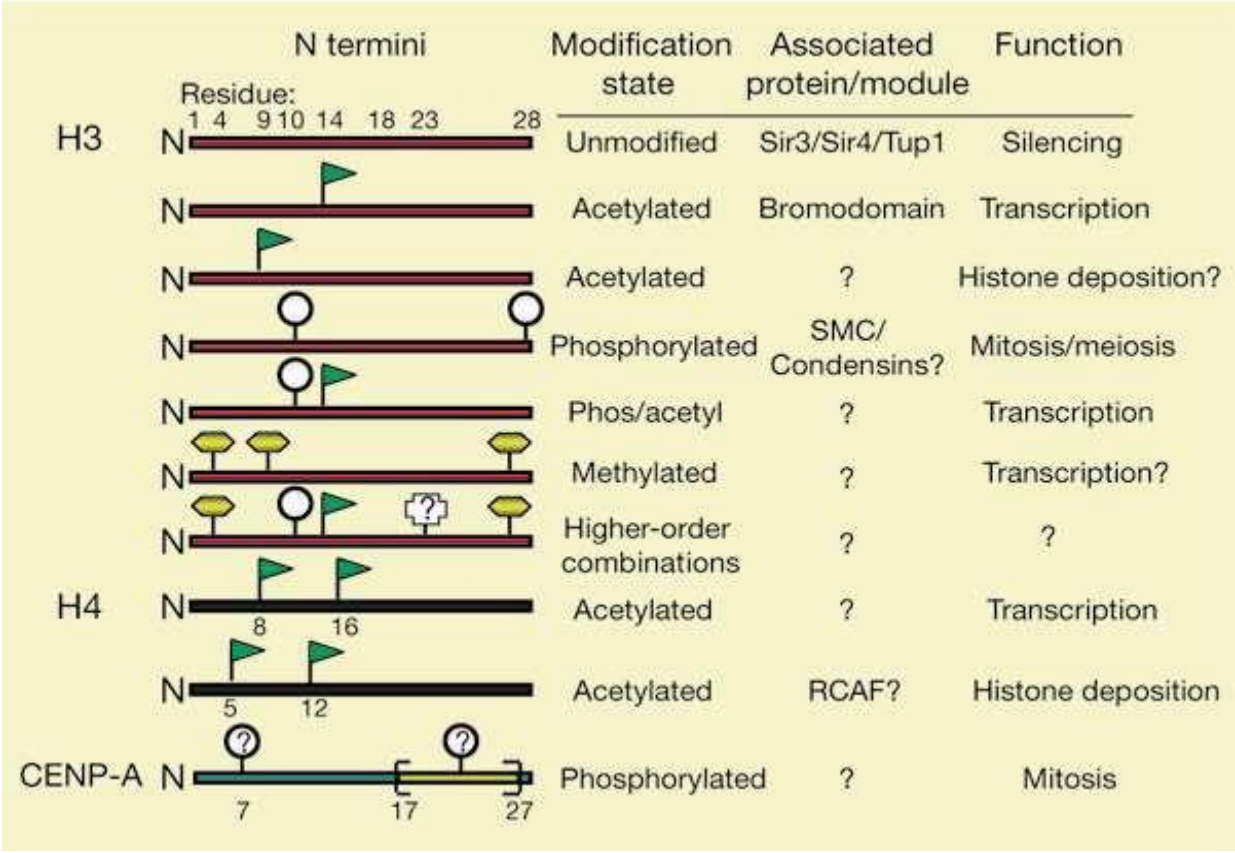
Crosstalk among Modifications



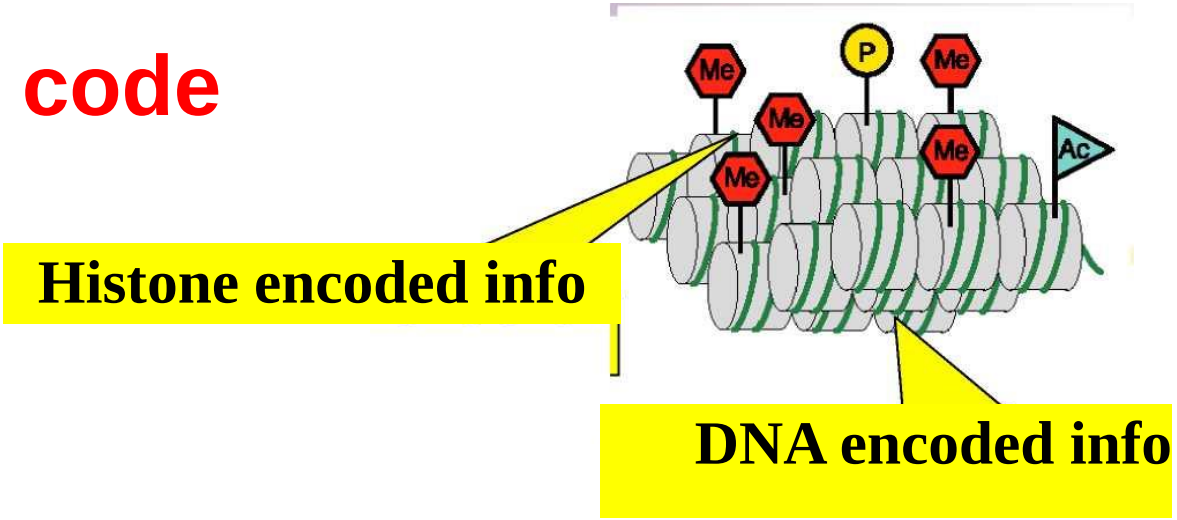
One complex may contain both demethylase and methyltransferase targeting different residues with opposing functions

Histone Code

- **The histone tail modifications can occur in various combinations**, many of which have specific meaning- **HISTONE CODE.**
- This code is read by specific protein complexes which contain protein modules which recognise specific histone marks and bind to them.

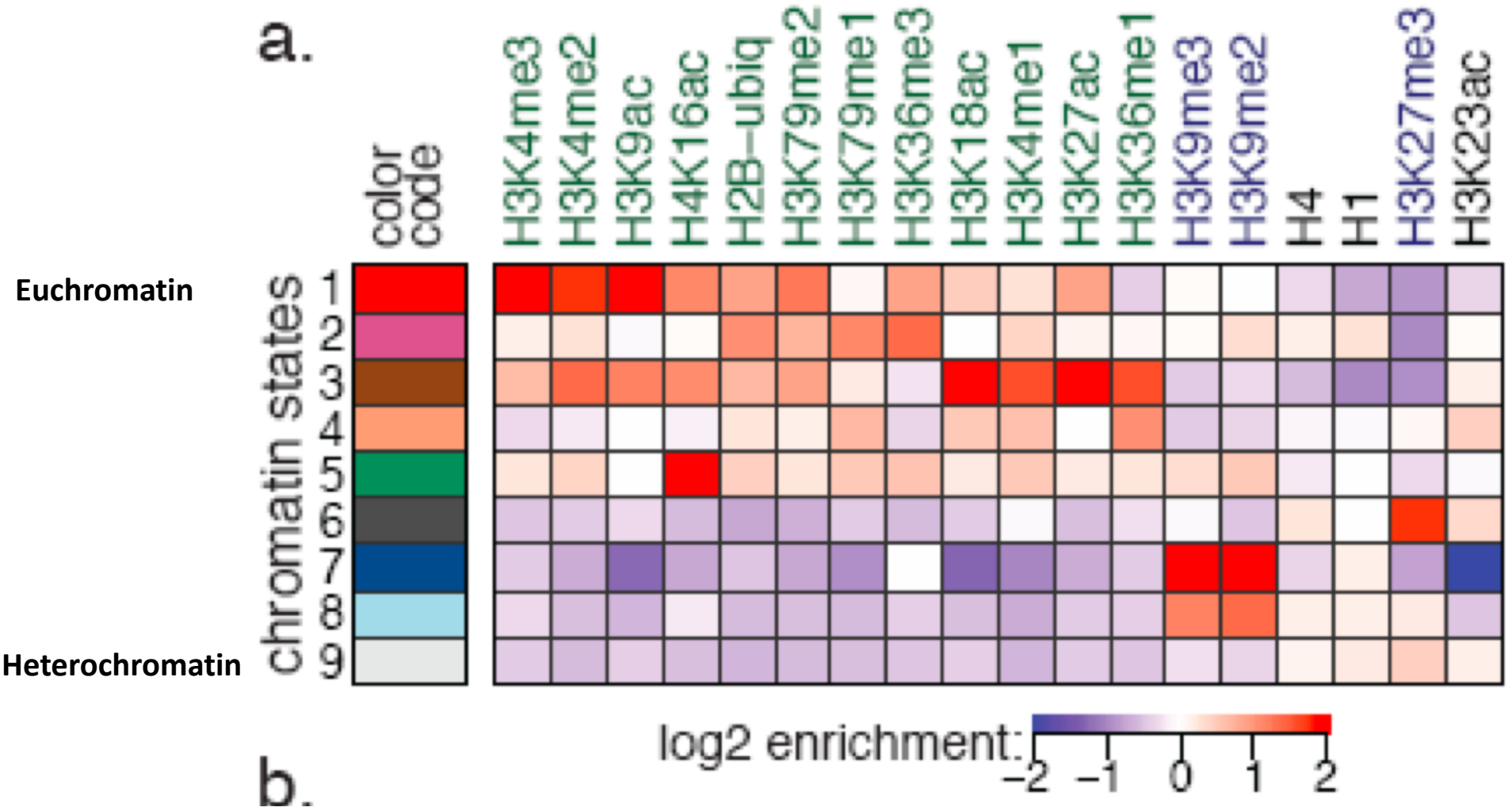


The histone code



- **Modifications of histone tails in specific patterns represent an encoded information extending the information content of the genome beyond the DNA sequence**
- **This hypothesis predicts that**
  - Distinct modifications of the histone tails will induce interaction affinities for chromatin-associated proteins
  - Modifications may be interdependent generate various combinations
  - Local concentrations and combinations of differently modified nucleosomes determine qualities of higher order chromatin

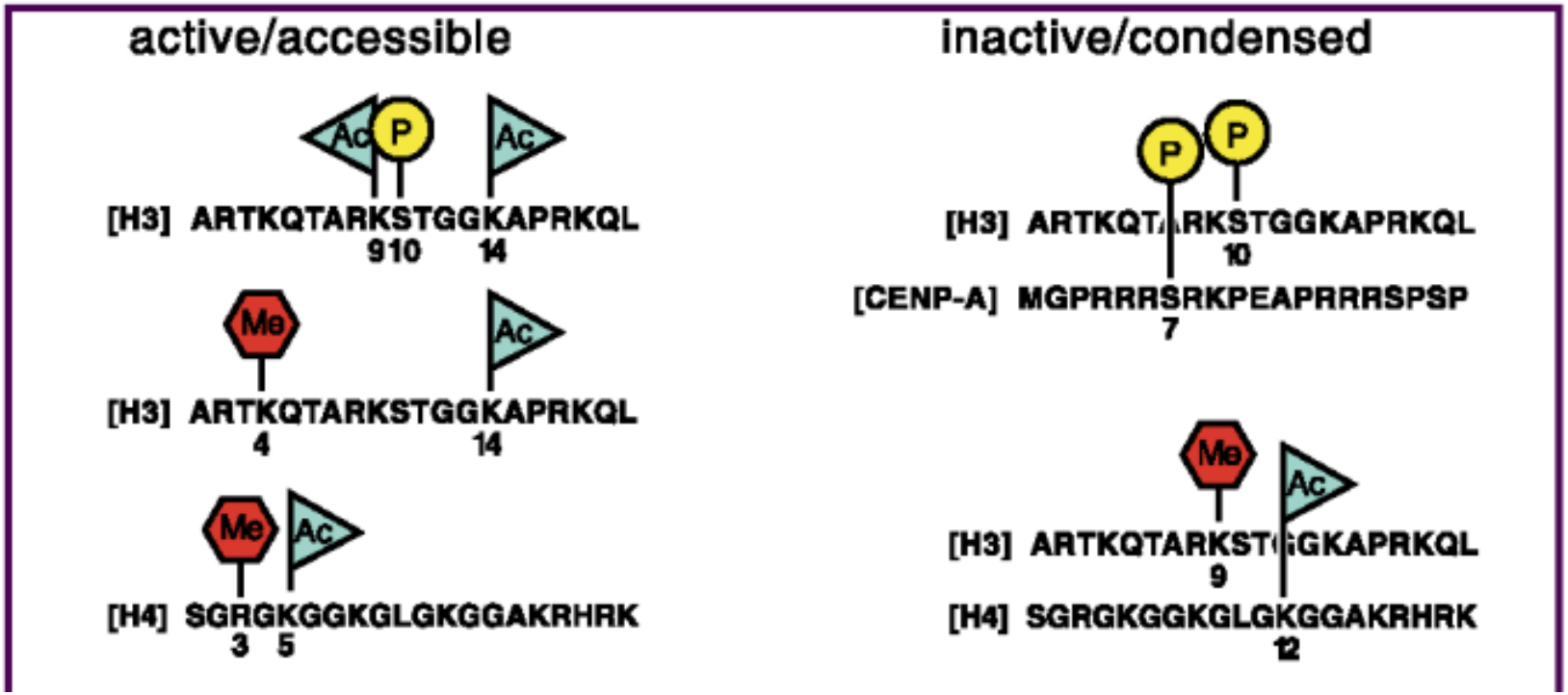
So…is there a histone code?



Kharchenko et al., Nature, 2011.

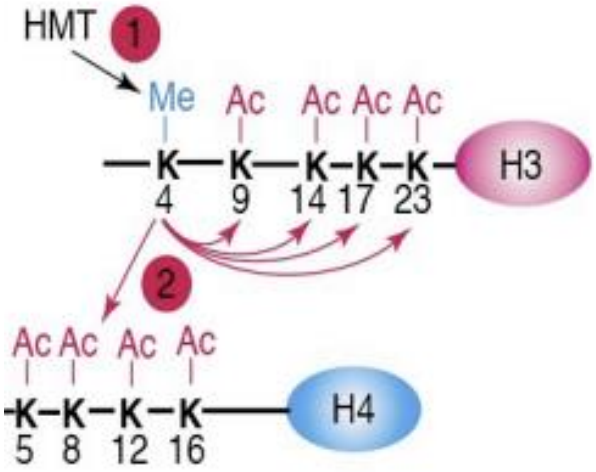
Combinatorial possibilities large - a language?

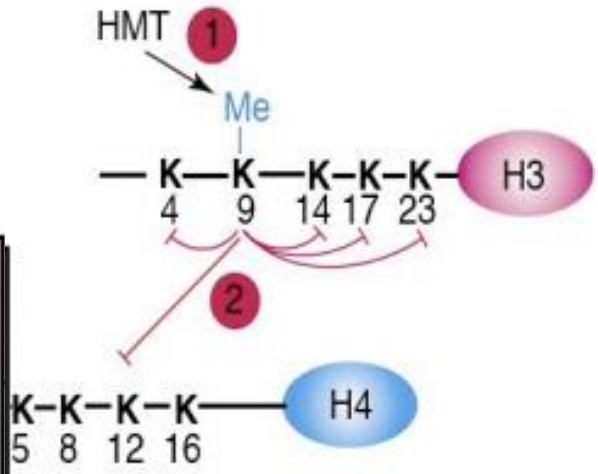
- Acetylated = acitve, deacetylated = inactive
  True but probably too simple
- Many combinations - link to active/inactive chromatin complex
- Number of combinations very high

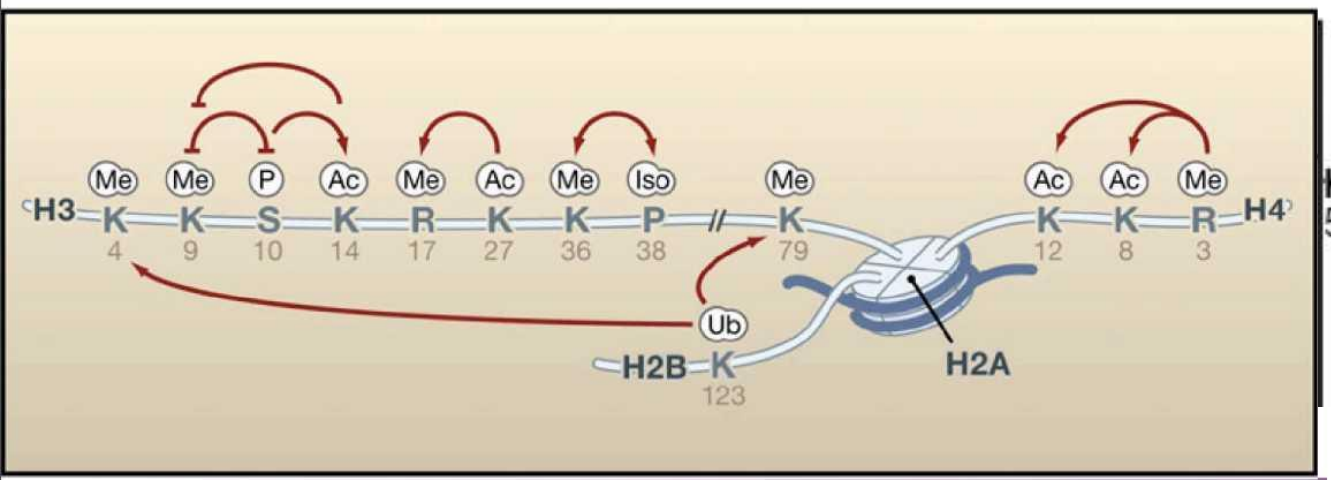


Synergistic or antigonistic modifications of histones

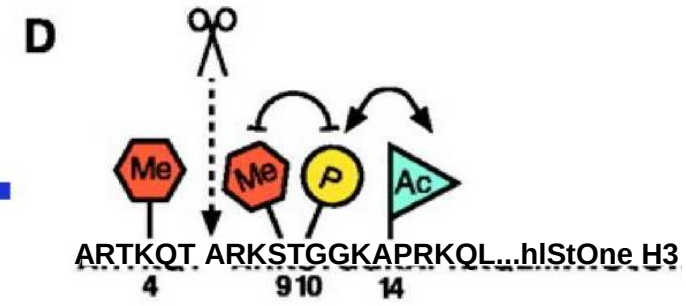
- One modification can enhance or inhibit another modification



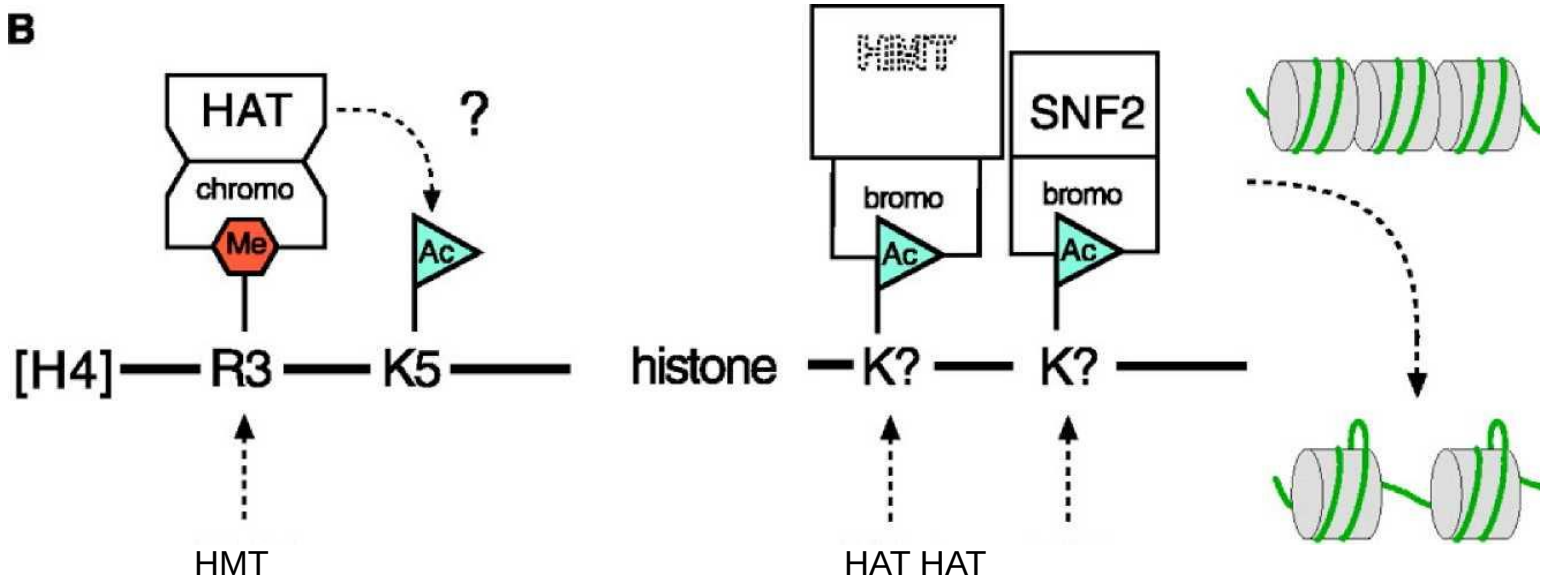




Cross-talk between histone modifications







Science 2001, 293, 1074-1079.

Summary: Histone PTMs and readout

1. **Type of modification**
   - Which amino-acid
   - Number of modifications (me)
2. **Position in genome**
   - Promoter: H3K36me, H3K9me are repressive
   - Coding region: H3K36me, H3K9me are activating and prevent cryptic initiation of transcription in ORF
3. **Other histone modifications**
   - combinatorial (occur together)
   - H3K4me + H3K9me: transcriptional activation
   - H4K20me + H3K9me: heterochromatin formation
   - H3K27me + H3K4me: “bivalent” mark in stem cells
4. **Size of histone modification domain**
   - large: heritable (can be copied more easily)
     - H3K27me can recruit PRC2 has H3K27me3 activity
     - H3K4me recruits WDR5 (MLL thrithorax): H3K4me
5. **Cycles of modifications**
   - H2Bub → H2B required for transcriptional elongation

2c. ATP-dependent remodelling

Chromatin remodeling complexes alter the positions and compositions of nucleosomes

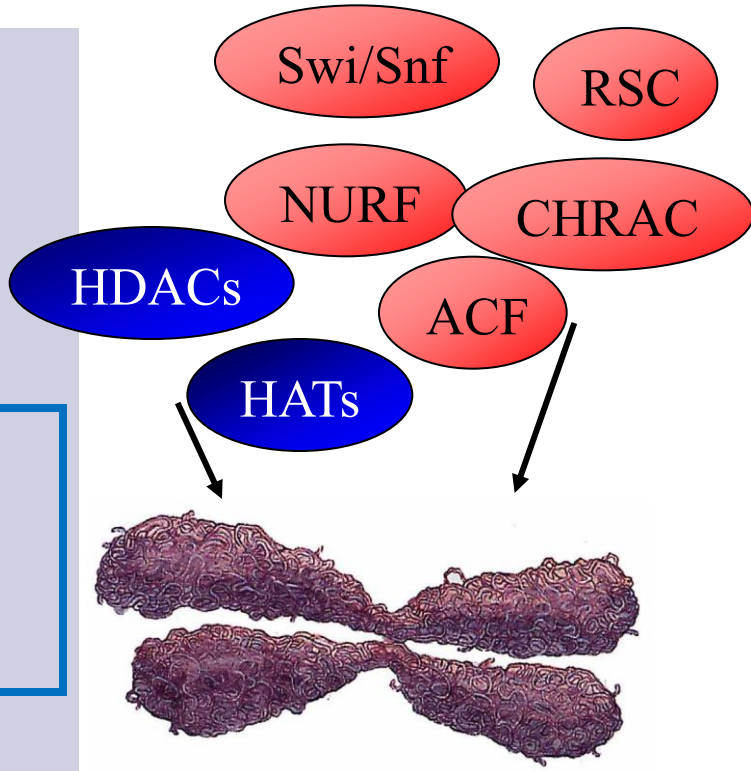
- ✓ A key role of some transcriptional activators is to orchestrate changes in chromatin structure from the **closed to the open conformation by altering nucleosomes.**

- ✓ One way to change chromatin structure is through **ATP-dependent chromatin remodeling.**

- ✓ All remodeling complexes **have a catalytic ATPase subunit** that is similar to other motor proteins, called **DNA translocases,** that move along the DNA.

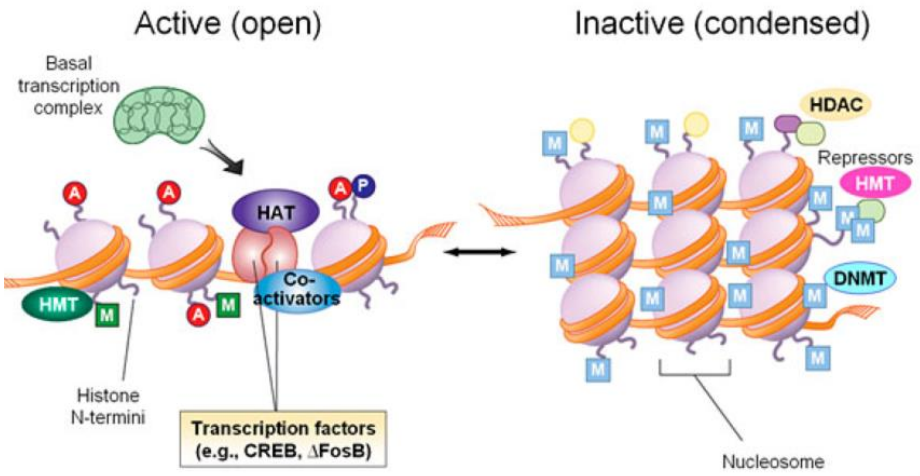
Changing the chromatin-template to help transcription factors

- 1. Opening of chromatin through directed **modication of histone tails** (acetylation and methylation)
  - Histon-modification by HAT and HMT activity
  - Basis for the histone code hypothesis
- 2. Opening of chromatin through directed **nucleosome mobilization**
  - ATP-dependent process
- 3. Positioning of nucleosomes creates promoters with different requirement for remodeling



Histone Modifiers

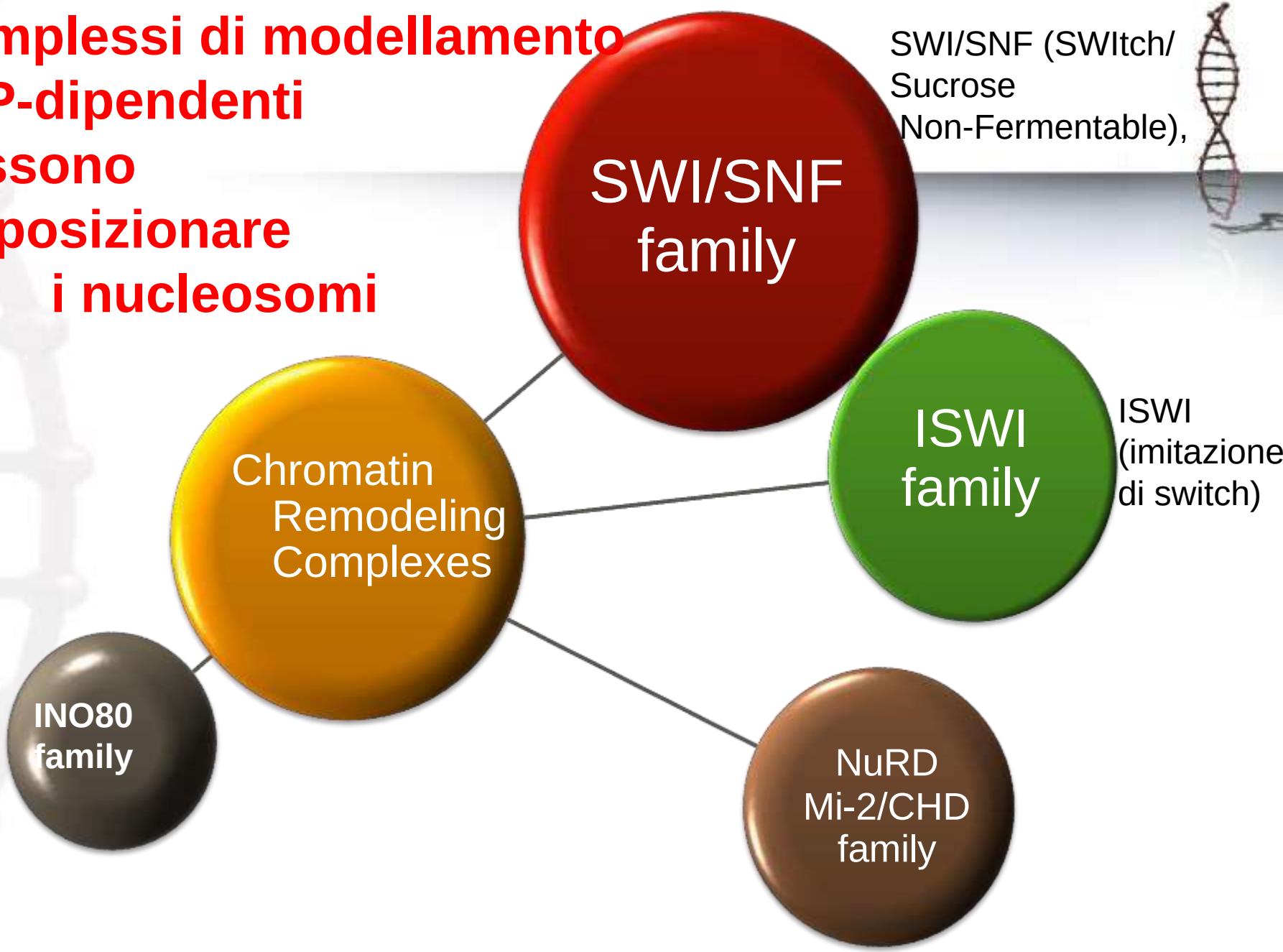
- **Do not bind to DNA themselves**
  - Can be recruited by:
    - **Histone modifications (through chromodomains, bromodomains, etc.)**
    - Transcription factors
    - RNA (fission yeast, mammals, plants)
    - DNA damage



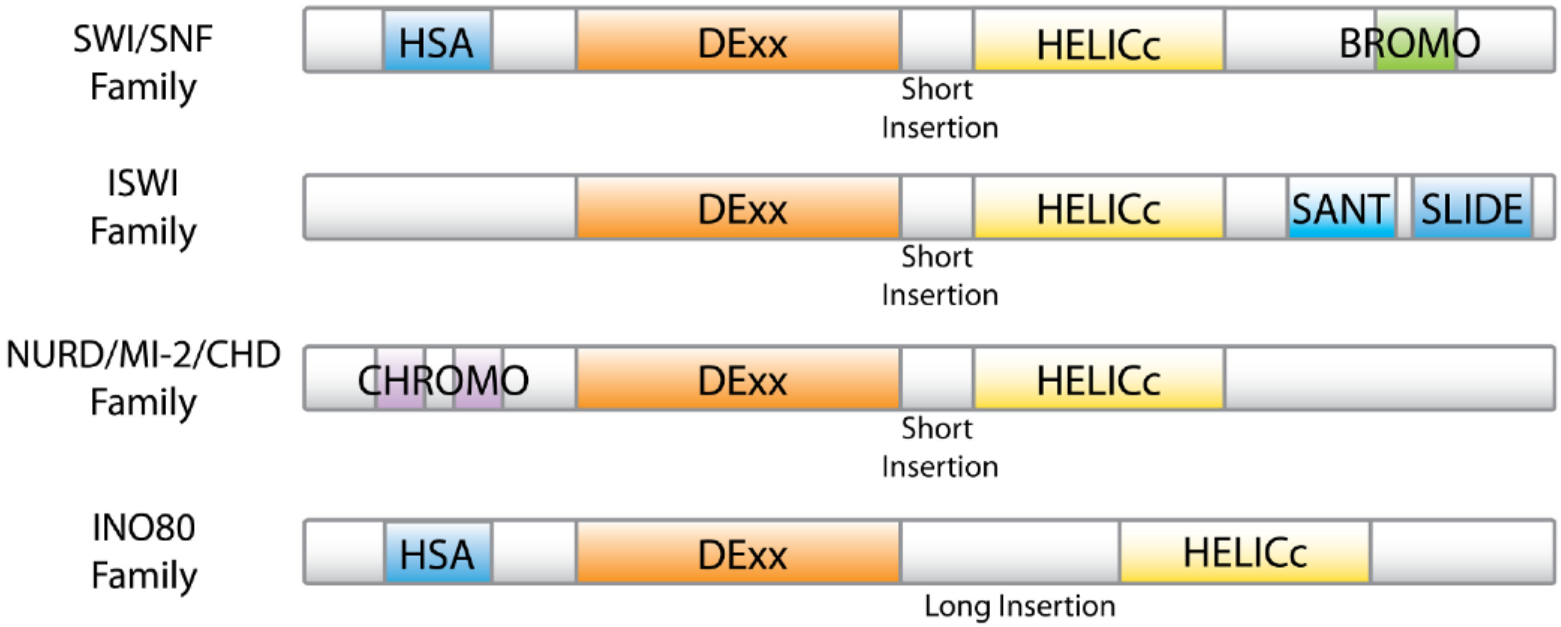
- **Act as transcriptional co-regulators**

- **Enhance activities of transcriptional repressors or activators**
  - Co-repressor: ex. HDACs
  - Co-activator: ex. HATs

Complessi di modellamento ATP-dipendenti possono riposizionare i nucleosomi



Summary of different classes of nucleosome remodeling ATPases and reactions they catalyze.



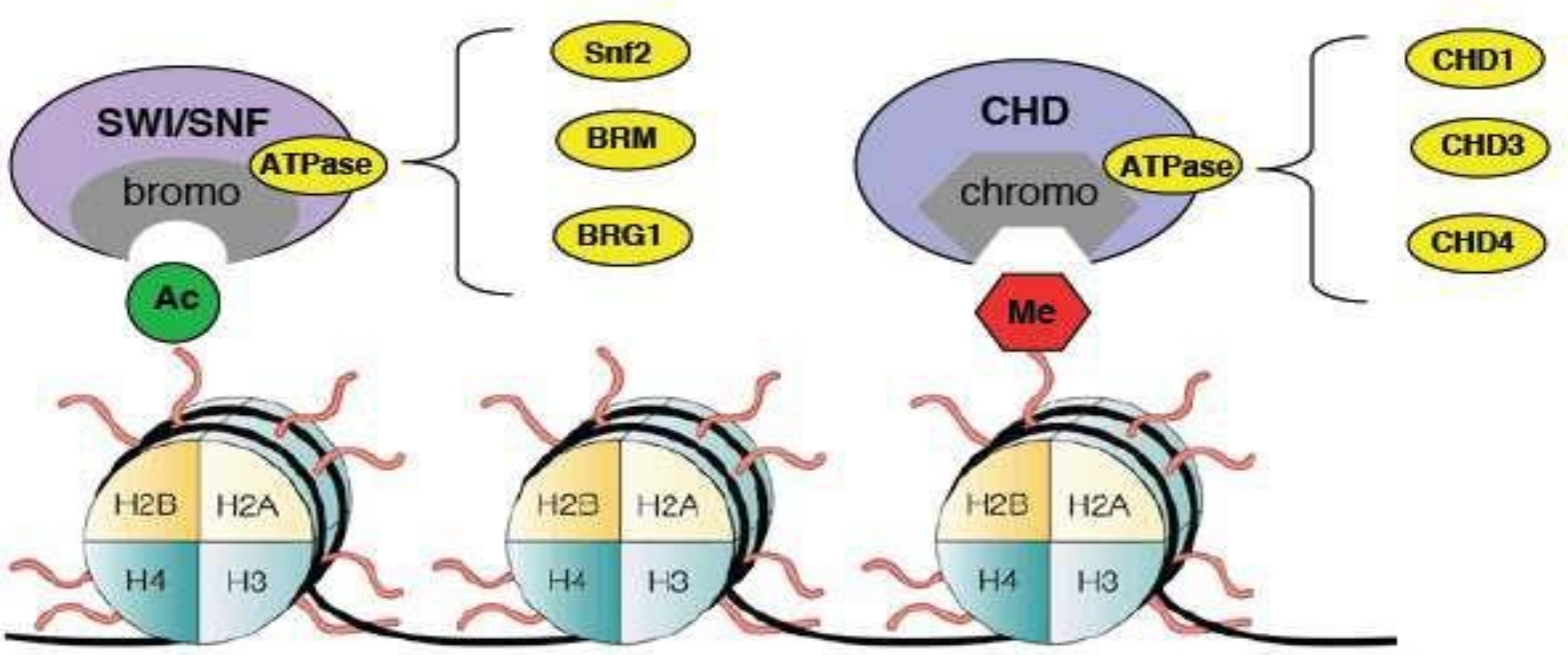
The ATPase subunit of all the remodeling complexes **belongs to the superfamily II helicase group**. The ATPase always contains a DExx and a HELICc domain, spaced by a linker. The remodelers are classified into different families based on the presence of additional domains on their ATPase subunits. The SWI/SNF family contains a HSA domain, involved in actin binding, and a bromodomain important for the binding of acetylated lysines. The ISWI family contains the SANT and SLIDE domains, important for histone binding. The CHD/NURD/ Mi-2 family contains a tandem Chromo domain, also used for histone binding. The INO80 family, like the SWI/SNF family, comprises a HSA domain but it is also characterized by the presence of a longer insertion between the DExx and the HELICc domains.

ATP-dependent chromatin remodeling complexes

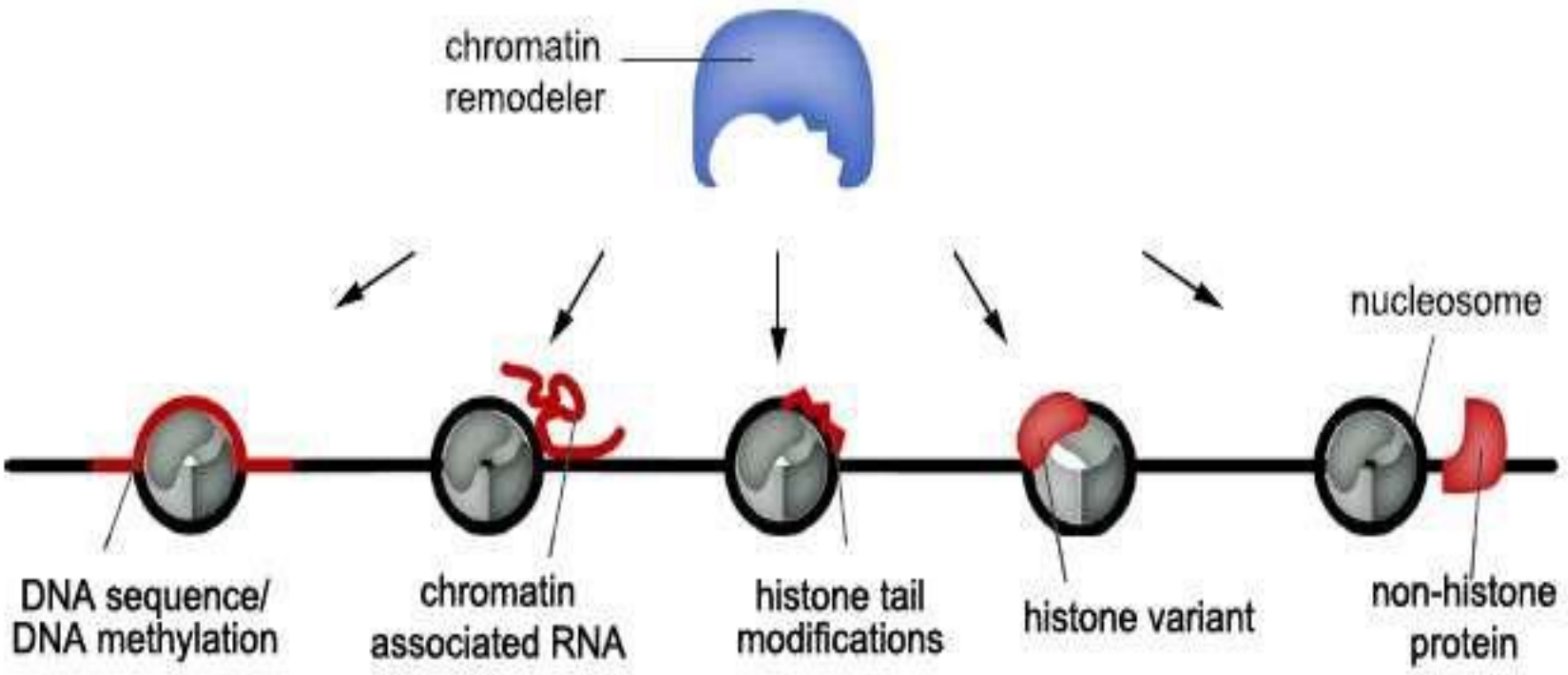
main families:

→ **SWI/SNF:** enzyme **contains** **bromo-domain that binds to acetylated histone**

→ **CHD: enzyme c**ontains **chromo-domain that binds to methylated histone**



Target location signals and translocation reaction scheme for chromatin remodelers.



Mechanisms of Chromatin Remodeling

- Nucleosome Sliding

One

- eject histone octamers

Two

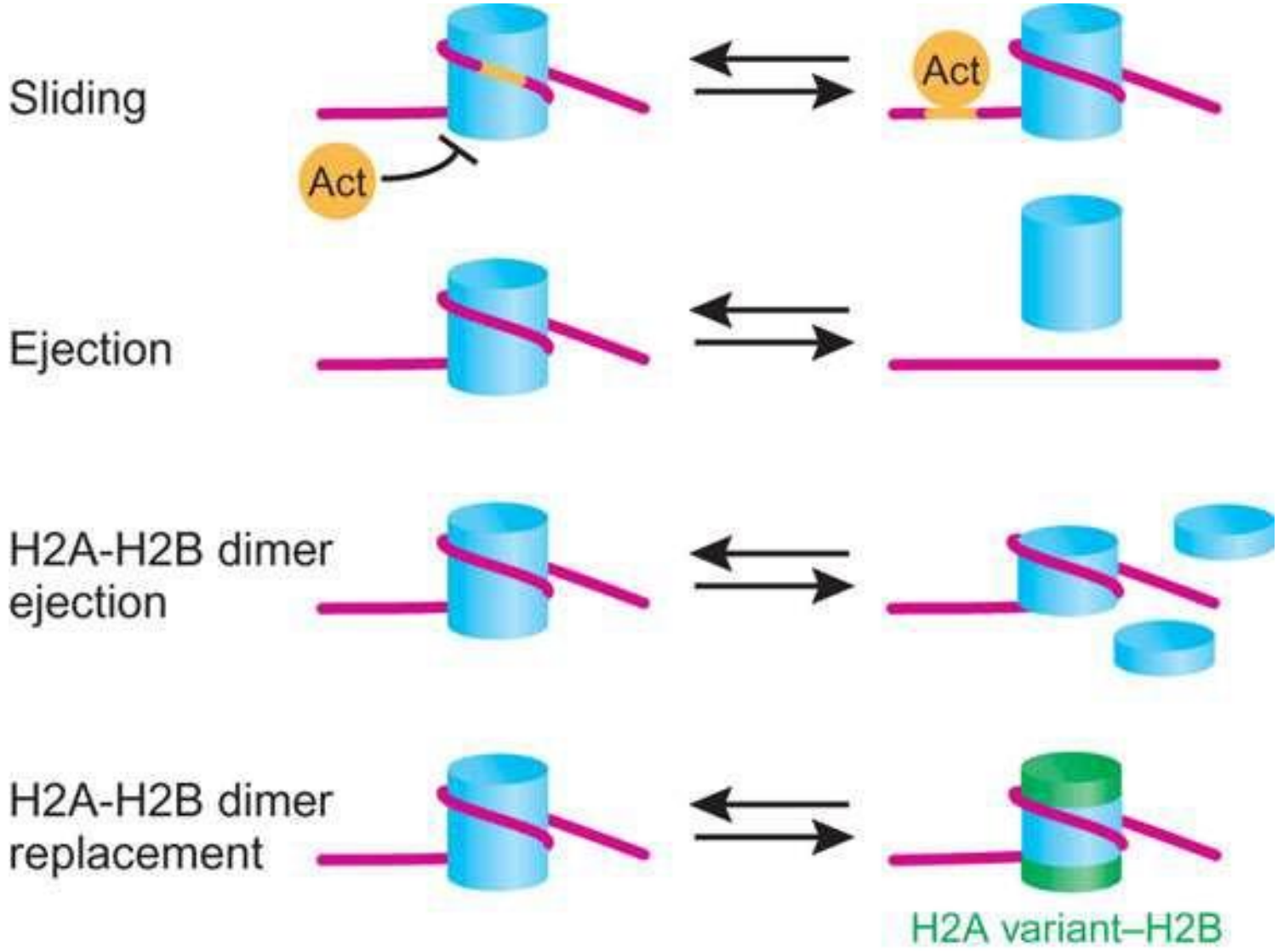
- Remove H2A-H2B dimers

Three

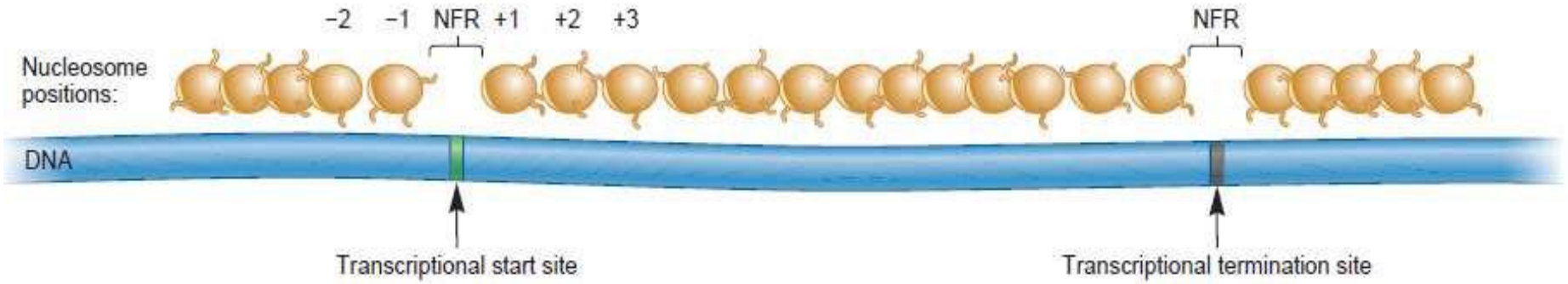
- replace dimers

Four

Mechanisms of Chromatin Remodeling

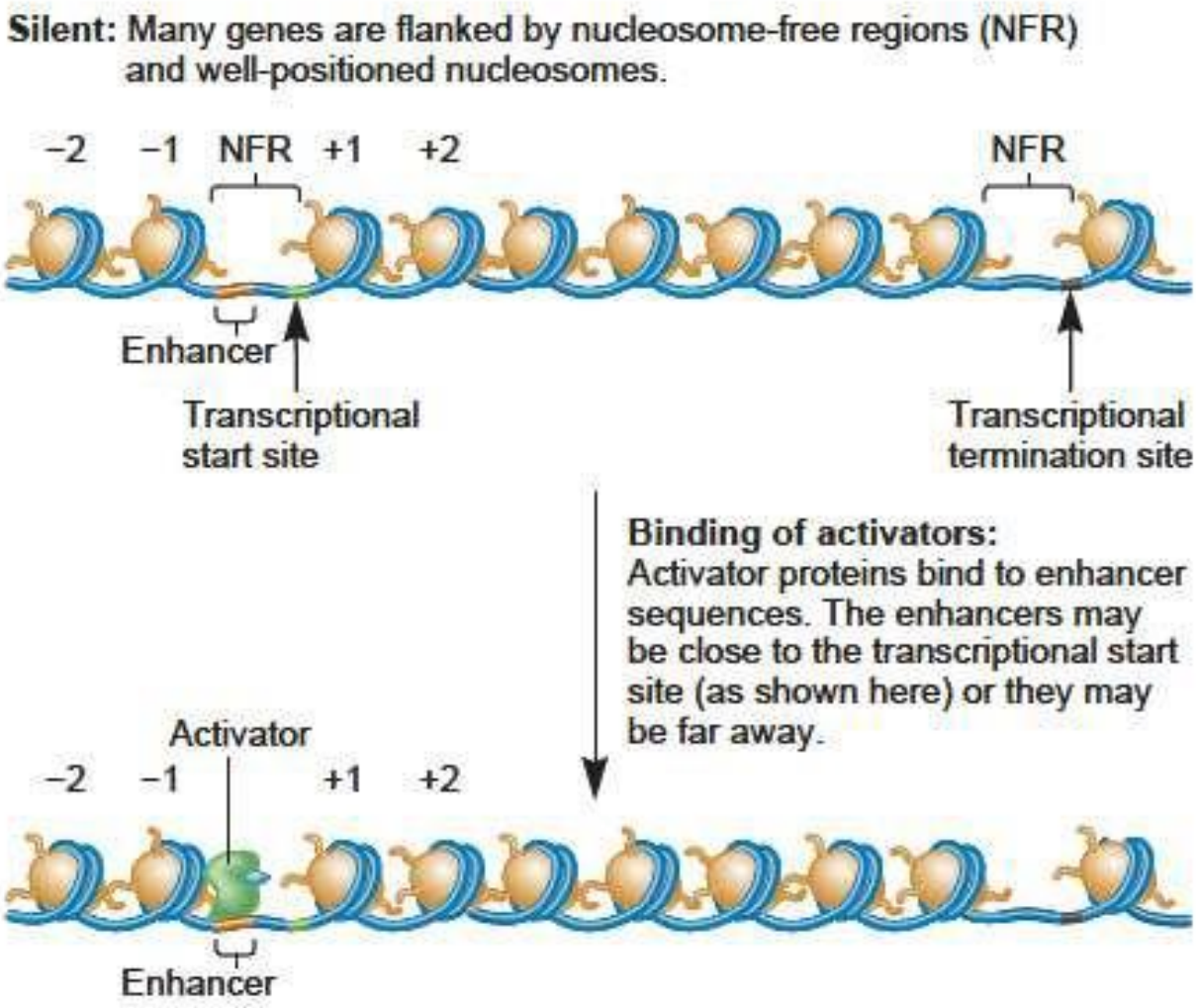


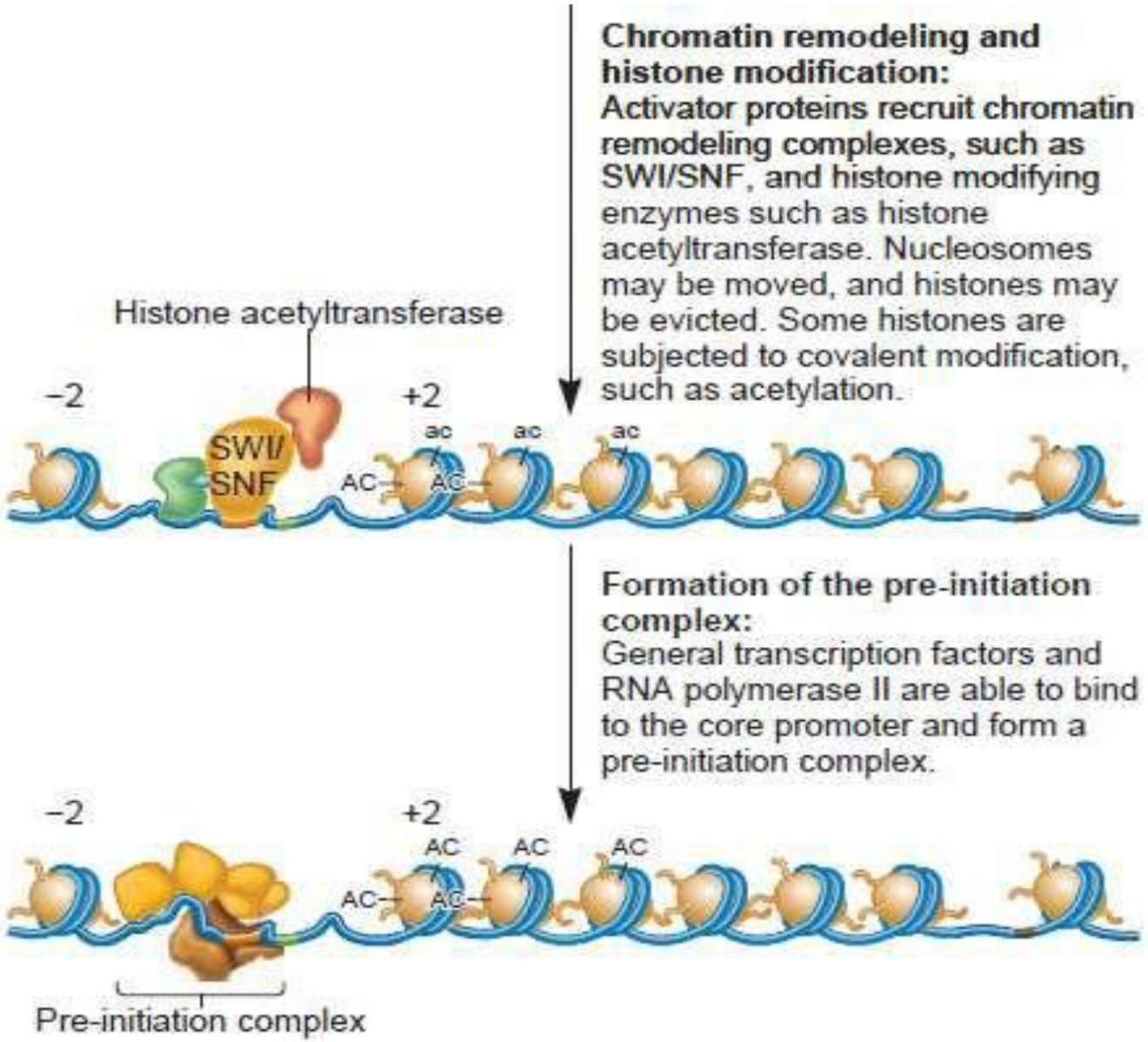
Eukaryotic Genes Are Flanked by Nucleosome-Free Regions (NFR) and Well-Positioned Nucleosomes

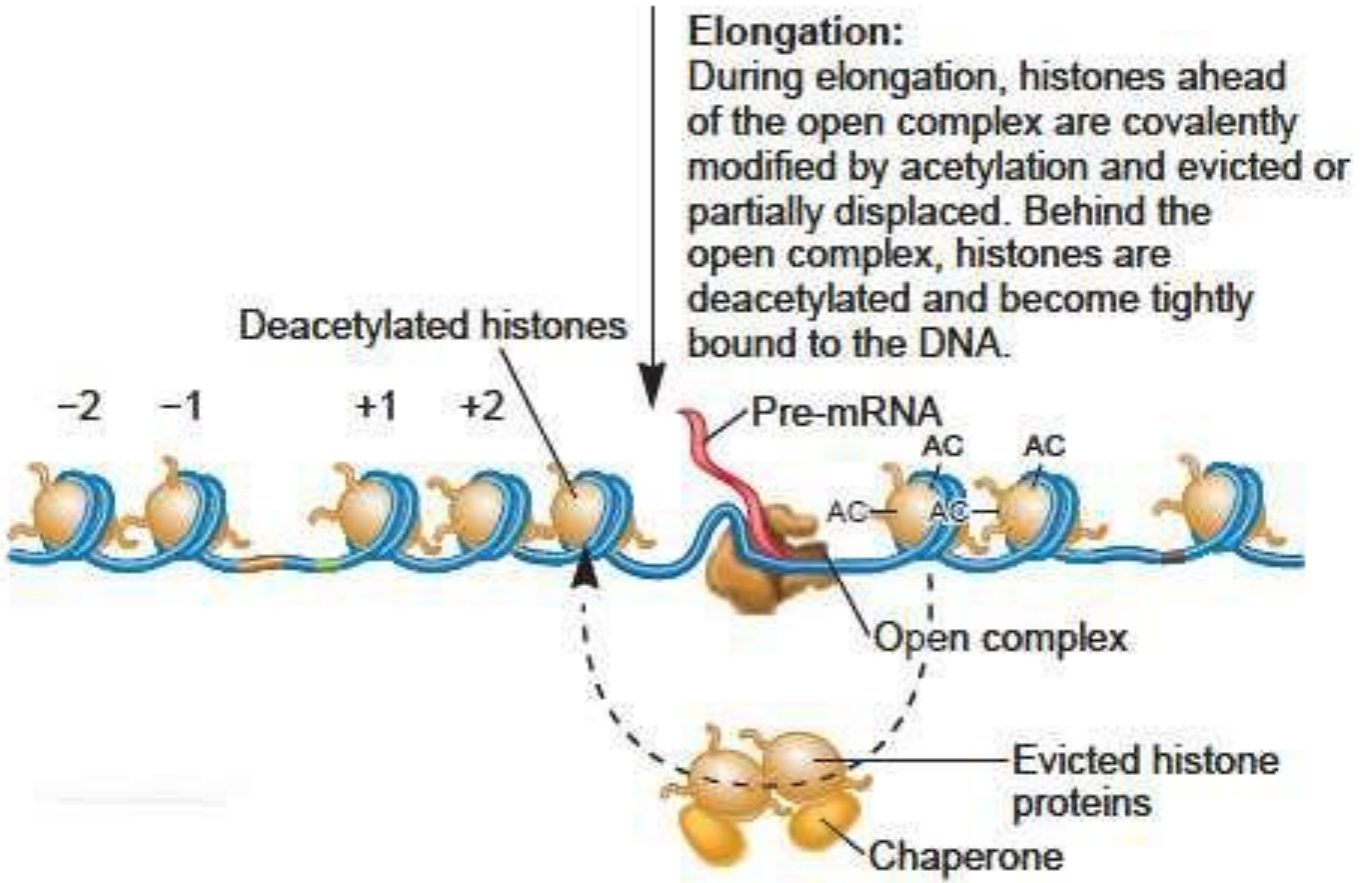


- A nucleosome free region (NFR) is found at the beginning and end of many genes.
- Nucleosomes tend to be precisely positioned near the beginning and end of a gene, but are less regularly distributed elsewhere.

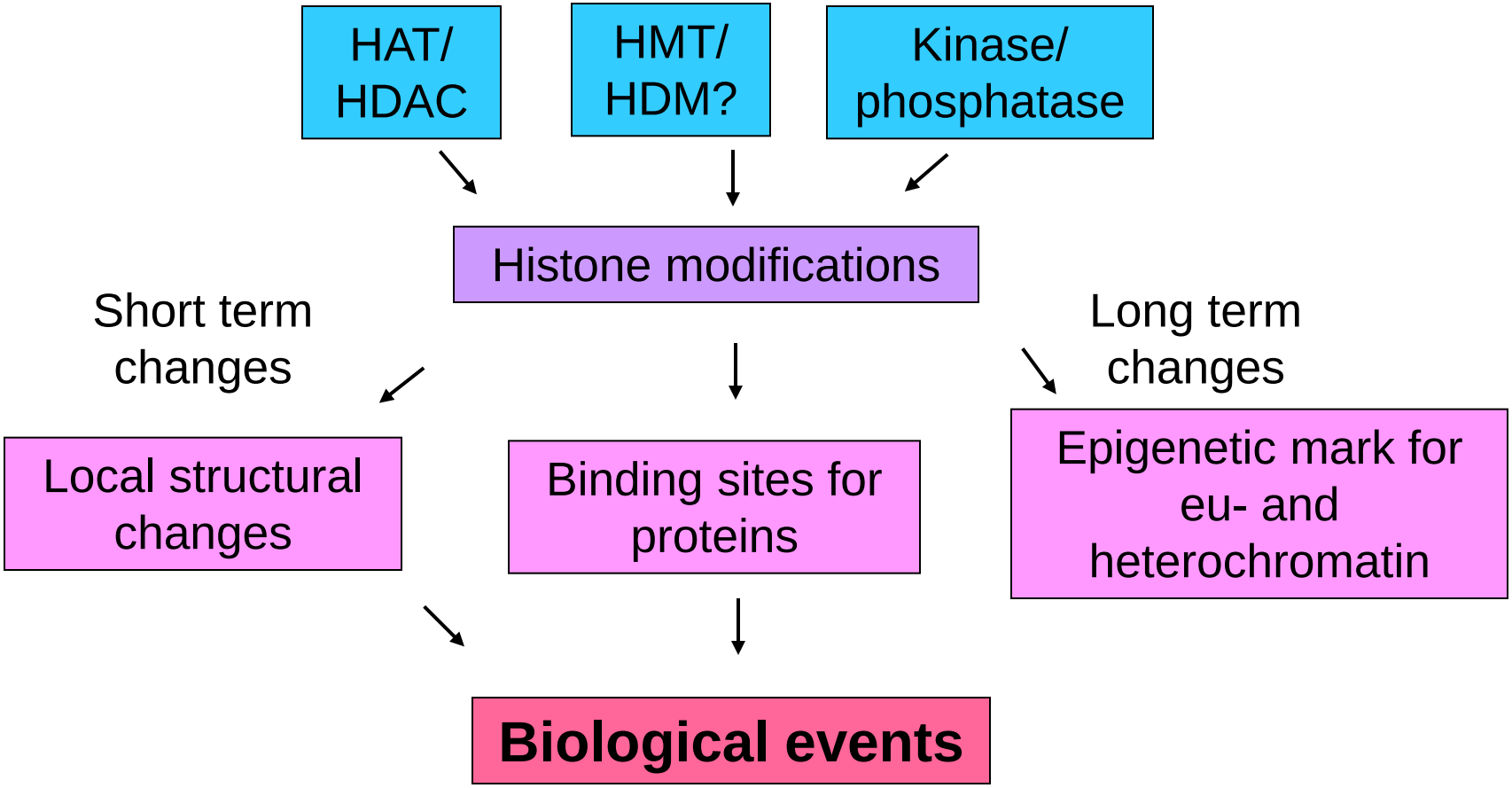
Transcriptional Activation Involves Changes in Nucleosome Locations, Composition, and Histone Modifications




Chromatin remodeling and histone modification:
Activator proteins recruit chromatin remodeling complexes, such as SWI/SNF, and histone modifying enzymes such as histone acetyltransferase. Nucleosomes may be moved, and histones may be evicted. Some histones are subjected to covalent modification, such as acetylation.
Histone acetyltransferase
−2
+2
SWI/
SNF
ac
ac
ac
AC
AC
Formation of the pre-initiation complex:
General transcription factors and RNA polymerase II are able to bind to the core promoter and form a pre-initiation complex.
−2
+2
AC
AC
AC
AC
AC
Pre-initiation complex

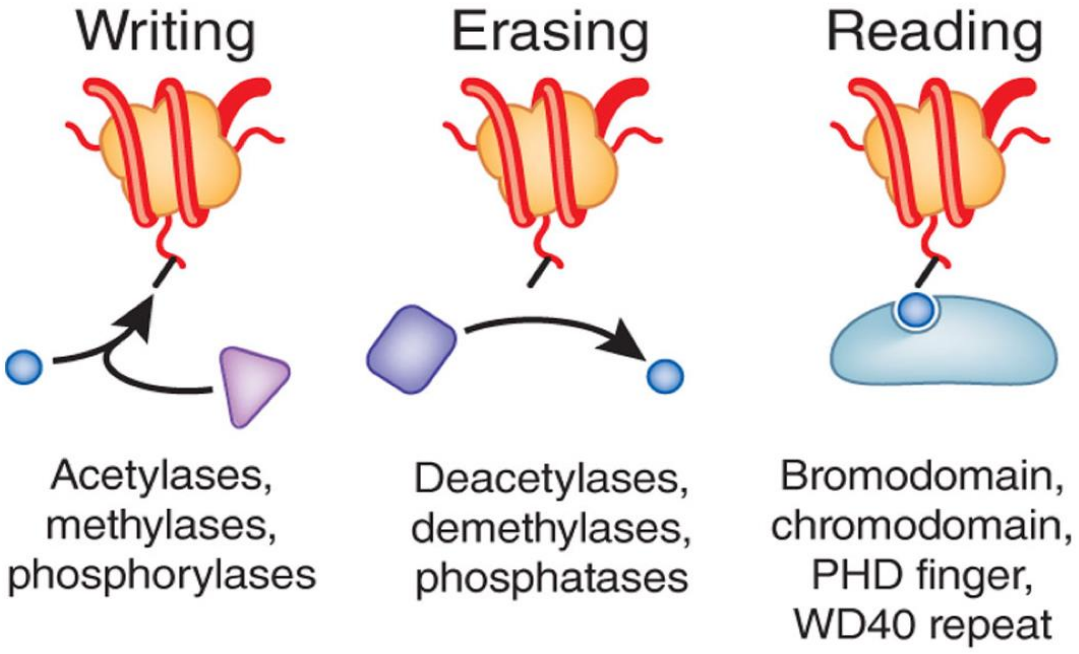

Elongation:
During elongation, histones ahead of the open complex are covalently modified by acetylation and evicted or partially displaced. Behind the open complex, histones are deacetylated and become tightly bound to the DNA.
Deacetylated histones
−2
−1
+1
+2
Pre-mRNA
AC
AC
AC
AC
Open complex
Evicted histone proteins
Chaperone

Mendel's gene is more than just a DNA moiety!!



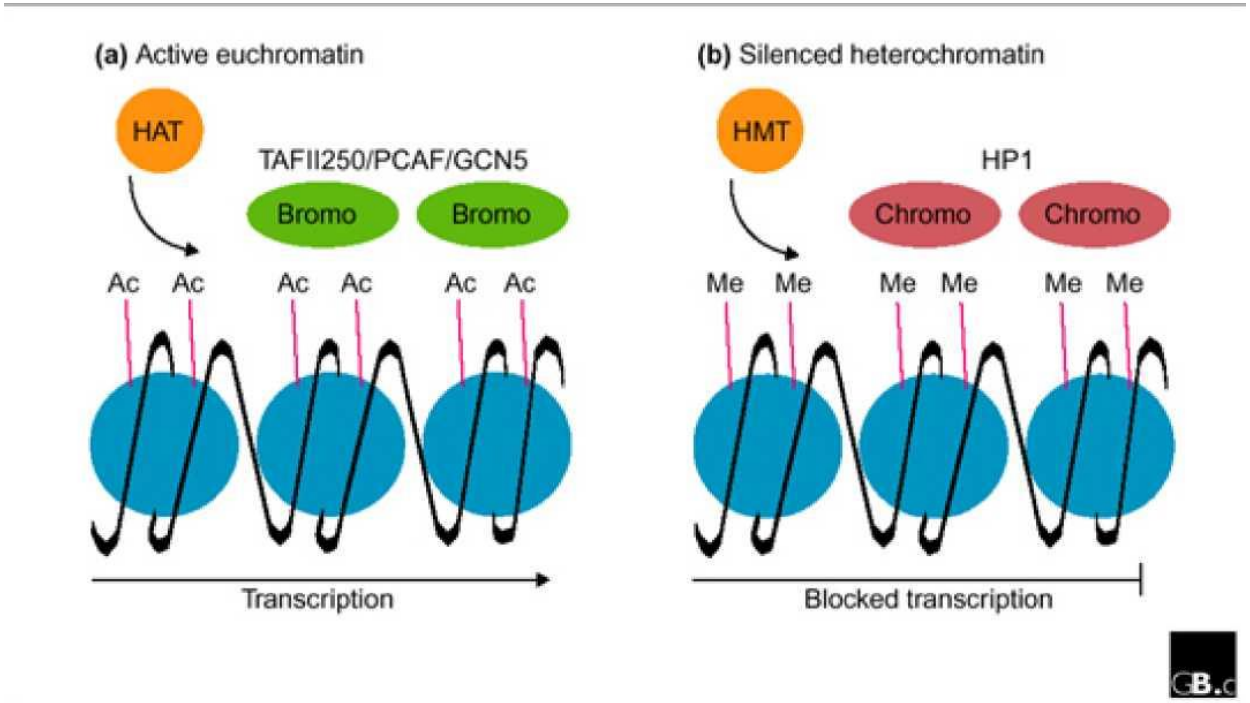
Histone Modifications and Modifers

- **Writers**: enzymes that add a mark
- **Readers**: proteins that bind to and “interpret” the mark
- **Erasers**: enzymes that remove a mark



Writing & Reading the histone code

- The **authors:** HMTs, kinases, HATs, PPTases, HDACs, HDM?
- The **readers**: Chromo- and bromo domain proteins.



Genome Biology 2001,2(4): reviews003.1.6

Table 1 | Chromatin modifications

Mark*	Transcriptionally relevant sites†	Transcriptional role‡
DNA methylation		
Methylated cytosine (meC)	CpG islands	Repression
Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123§/120¶) H2A (119¶)	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30–38)	Activation/repression

*The modification on either DNA or a histone.
†Well-characterized sites with regard to genomic location for DNA methylation or residues within histones for PTMs.
‡Whether the epigenetic mark is associated with activation or repression.
§Yeast (*Saccharomyces cerevisiae*).
¶Mammals.

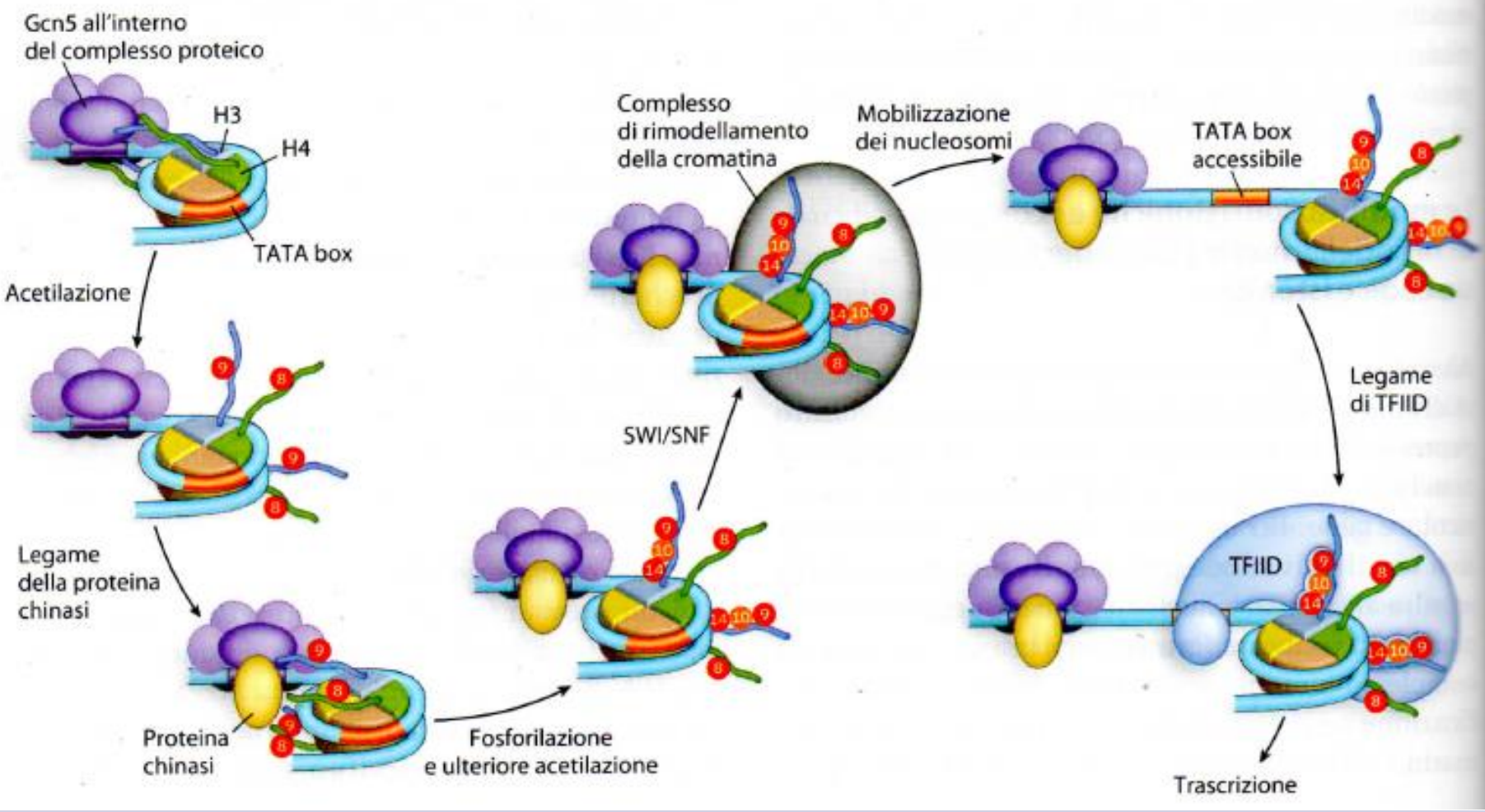


Histone Modifications and Modifers

Residue	Modification	Modiying Enzyme
Lysine	Acetylation Deacetylation	HAT HDAC
Lysine	Methylation Demethylation	HMT HDM
Lysine	Ubiquitylation Deubiquitylation	Ub ligase Ub protease
Serine/Threonine	Phosphorylation Dephosphorylation	Kinase Phosphatase
Arginine	Methylation Demethylation	protein R methyltransferase) PRMT Deiminase/Demethylase

- Others: Sumoylation (Lysine), ADP Ribosylation (Glutamate)

Un esempio di codice istonico.



- L'attivazione del promotore della IFN-β globina richiede una successione ordinata di eventi .
- Questi sono guidati dalle modificazioni degli istoni che avvengono in una sequenza temporale

3. Long non-coding RNA

- According to their modes of function, lncRNAs may be divided into four major types, namely
- **signals, decoys, guides, and scaffolds,** which are not mutually exclusive .
- lncRNAs with functions related to signals or decoys are involved in gene activation or suppression, respectively.
- lncRNAs that act **as guides recruit chromatin-modifying** enzymes to regulate gene expression in cis or in trans.
- lncRNAs that serve as scaffolds can recruit multiple proteins to assemble ribonucleoprotein complexes, which usually act on chromatin and/or modulate histone marks.
- Many lncRNAs possess more than one of the four charac-teristics listed above and therefore have integrative functions, as illustrated by the examples discussed below (Figure 4).

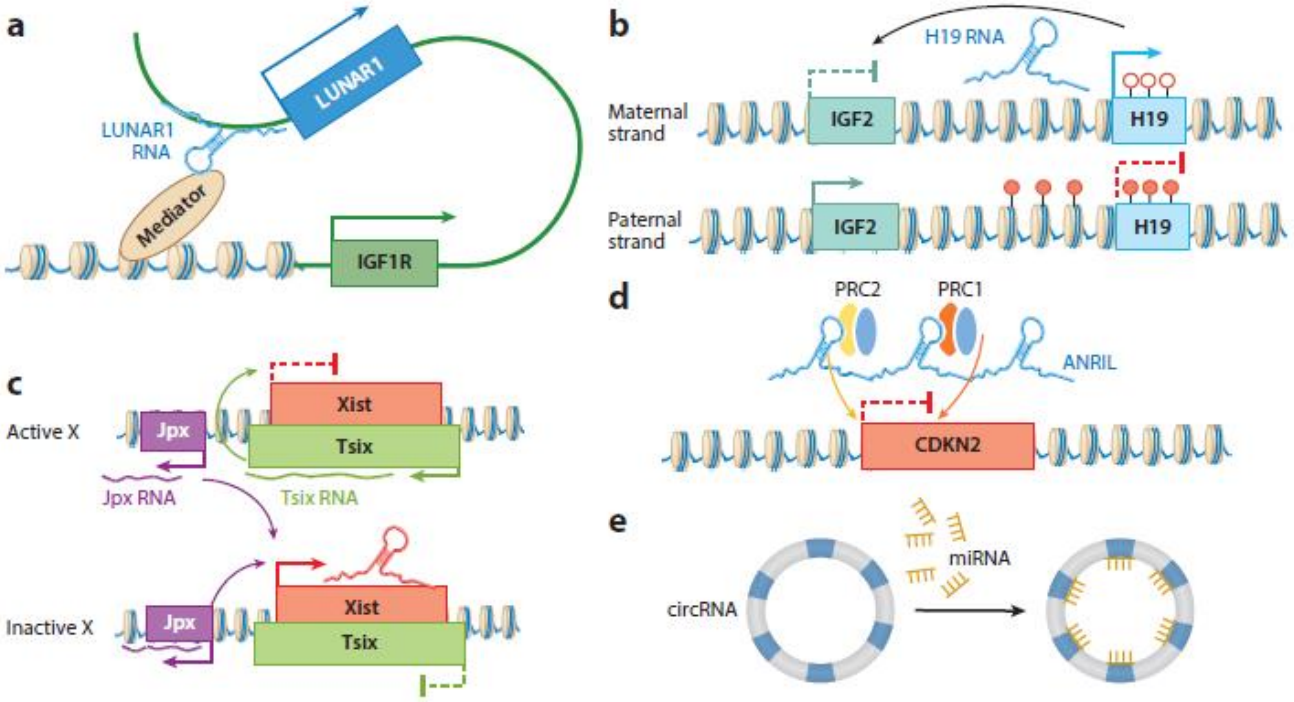
Modes of action of long noncoding RNAs (lncRNAs).

(a) The enhancer RNA (eRNA) LUNAR1 mediates **chromosome looping** between its own enhancer region and the nearby IGF1R locus via the Mediator complex, thereby promoting IGF1R transcription.

(b) The nonmethylation of the maternal H19 locus leads to the transcription of lncRNA H19.

H19 then represses the expression of IGF2 in cis, causing imprinting.

In parallel, methylation of the paternal H19 locus silences H19 transcription and thereby prevents H19 -mediated transcriptional suppression of IGF2.

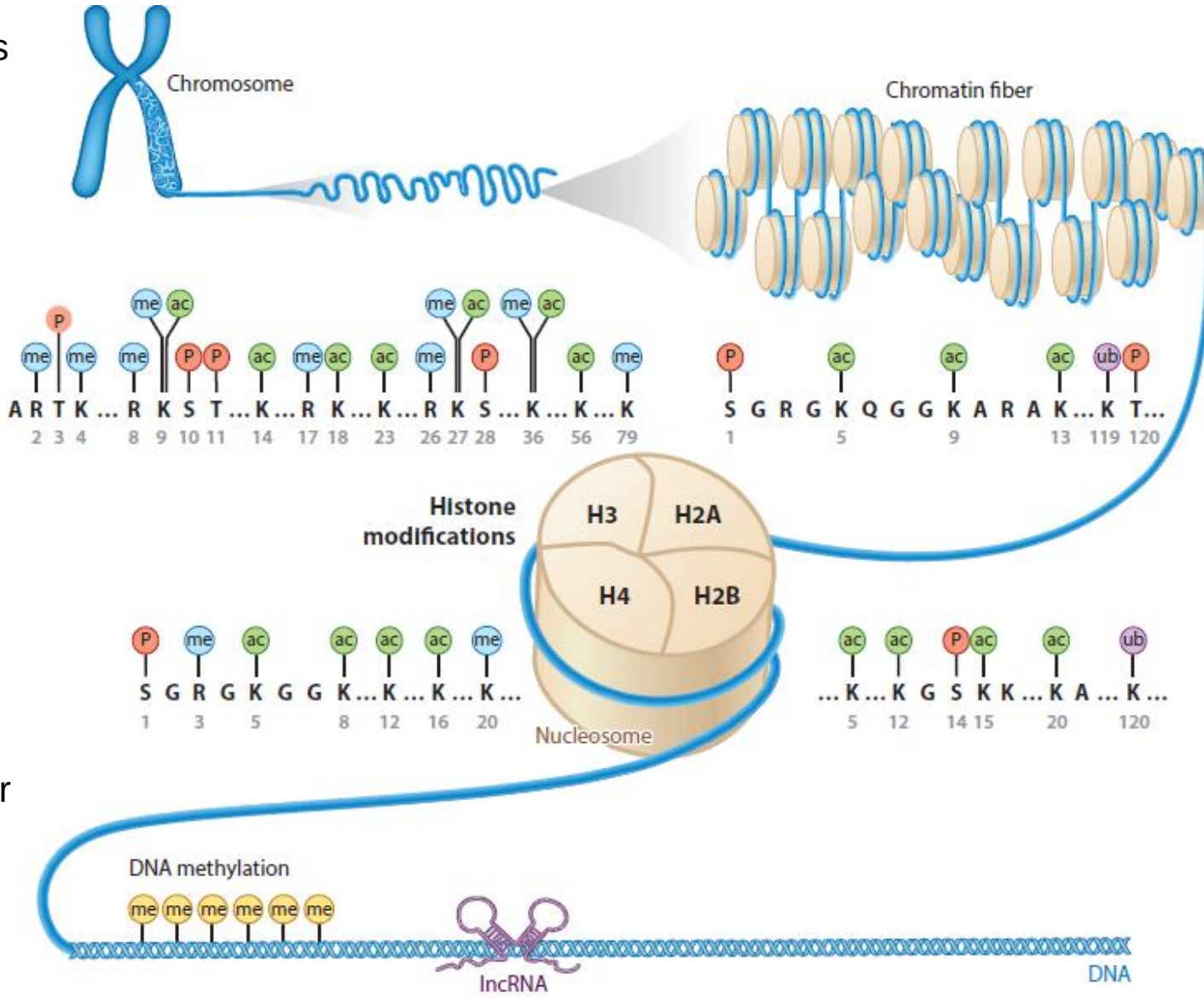


(c) The lncRNA Xist is **silenced on the active X chromosome in cis** by the antisense lncRNA Tsix. In the inactive X chromosome, Xist is activated both in cis and in trans by the lncRNA Jpx, which is encoded upstream of Xist but divergently transcribed. (d) Antisense noncoding RNA in the INK4 locus (ANRIL), encoded by the reverse strand of CDKN2, can recruit polycomb repressive complexes 1 and 2 (PRC1 and PRC2) to repress the transcription of CDKN2.

e) The circular RNA (circRNA) containing multiple antisense sequences of microRNAs (miRNAs) can act as an endogenous miRNA sponge to attenuate miRNA-mediated gene silencing.

Overview of chromosome structure and epigenetic modifications

- The binding of DNA to histone octamers allows DNA to be compacted into nucleosomes and sub-sequently into chromatin fibers and chromosomes.
- Chromatins are subjected to epigenetic regulations, including methylation of DNA and posttranslational modifi-cations of histones, including acetylation (ac), **methylation (me), phosphorylation (P)**, and **ubiquitination (ub).**
- Long noncoding RNAs (lncRNAs), through their interaction with DNA, can serve as important epigenetic modifiers to regulate transcription.



Take-Home Message –

- Chromatin organizes the genome of eukaryotic cells
  - into a **nucleosomal structure**
  - that facilitates the imposition of epigenetic information and assists in regulating the DNA sequence.
- The **nucleosomal structure can be modified** through
  - histone variants,
  - posttranslational histone modifications,
  - and DNA modifications that act combinatorically and synergistically.
- Specific enzymes that establish histone modifications can be considered **writers** of **epigenetic information.**
  - **readers** are proteins with affinity for histone marks that can recruit protein complexes with additional functions to chromatin.
  - **Erasers** are enzymes that remove chromatin marks and, thus, ensure that epigenetic information is reversible.

Take-Home Message Cont)

- **Folding of the linear DNA** in the nucleus facilitates the compartmentalization of chromatin into domains with different functional properties.
- **Chromosomes are not randomly** distributed but are organized into discrete volumes called chromosome territories, whereby separation of active A and repressed B compartments can be observed.
- **Modifications of chromatin structure** and chromatin topology both contribute to establishing information along the sequence of the DNA that regulates the function of distinct genomic regions.
- The u**nderstanding of nuclear pathways** for chromatin regulation is a basis for the consideration of epigenetic mechanisms that will further be discussed in following chapters.

4. Epigenetic Inheritance

- Most epigenetic modifications, *i.e.* epigenetic marks, that are established in most tissues during an organism's lifetime are irrelevant with respect to the next generation.

  ★This is because epigenetic modifications **are normally erased at each generation.** For example, DNA methylation is typically removed (or possibly oxidized; Iqbal et al. 2011) during zygote formation and re-established through successive cell divisions during development.

  ★And also because **only epigenetic modifications of the mature gametes** (and not in other tissues) have the potential to contribute to the phenotype of the offspring, the next generation.

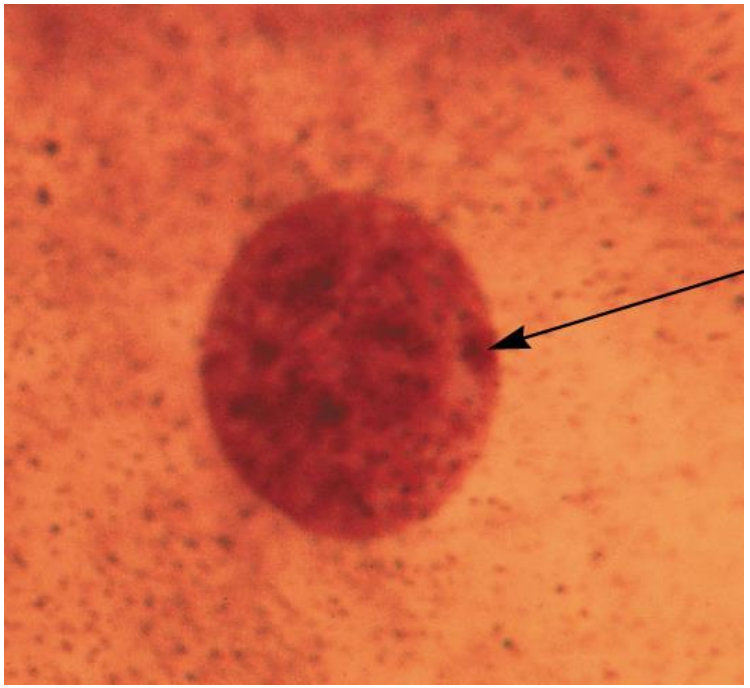
- **Only those epigenetic modifications that are heritable *via* the gametes (germ line) → lead to epigenetic inheritance.**

EPIGENETIC INHERITANCE

- Two types of epigenetic inheritance will be discussed
  - **Dosage compensation**
    - Offsets differences in the number of sex chromosomes
    - One sex chromosome is altered
  - **Genomic imprinting**
    - Occurs during gamete formation
    - Involves a single gene or chromosome
    - Governs whether offspring express maternally- or paternally-derived gene

DOSAGE COMPENSATION

- X chromosome inactivation
  - DNA in inactivated X chromosomes becomes highly compacted
    - A Barr body is formed
  - Most genes cannot be expressed

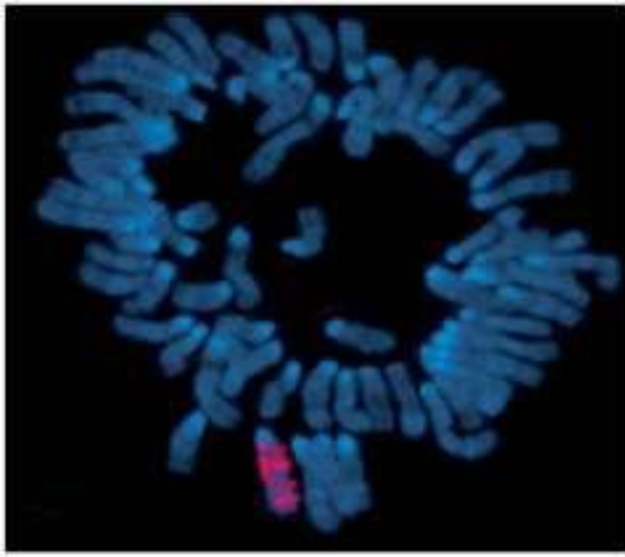


Non-coding RNAs

- A significant number of imprinted genes are transcribed to give a non-coding RNA.

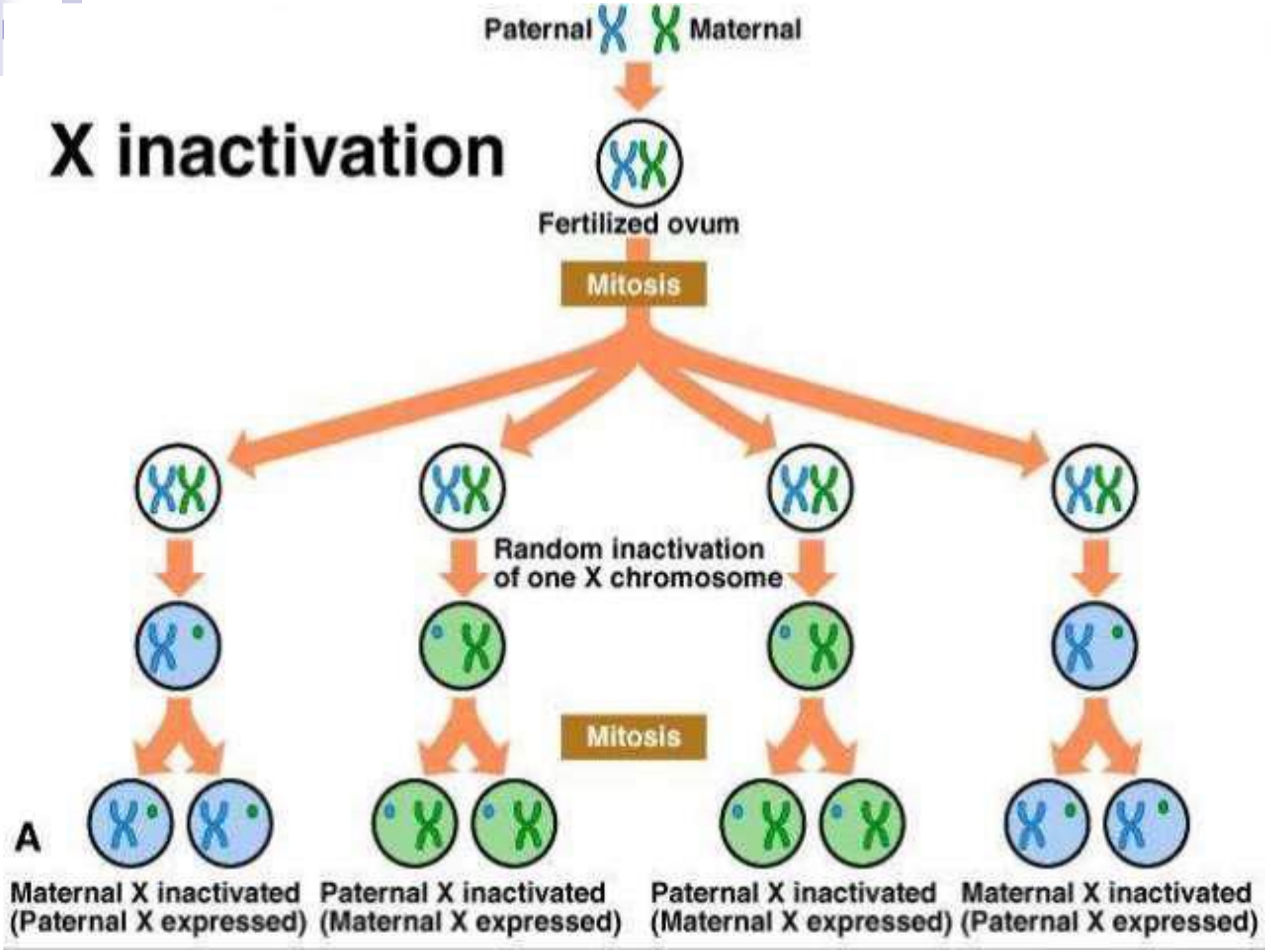
- Non-coding RNAs **include**
  - **antisense transcript,**
  - **small nucleolar RNAs (Sno RNAs),**
  - **micro RNAs,**
  - **pseudo-genes and**
  - **other RNA of unknown function**

- Random X-inactivation is associated coating of X chromosomes with Xist *(Plath K et al., 2003)*



X-INACTIVATION

- It is a process by which one of the copies of X chromosome present in female mammals is inactivated.
- Female mammals have two X chromosomes, X inactivation prevents having twice X chromosome gene products as males.
- X-inactivation is random and independent in the cells of early blastocyst.
- Once an X chromosome is inactivated it will remain inactive throughout the lifetime of the cell and its descendants in that organism.
- X-inactivation is reversed in the female gametes, so that all oocytes contain an active X chromosome.


Paternal
Maternal
X inactivation
Fertilized ovum
Mitosis
Random inactivation
of one X chromosome
Mitosis
A
Maternal X inactivated
(Paternal X expressed)
Paternal X inactivated
(Maternal X expressed)
Paternal X inactivated
(Maternal X expressed)
Maternal X inactivated
(Paternal X expressed)

Heterochromatin formation on Xi.

- The histone code is involved in **XCI** through histone modifications, such as H3K9me2, H3K27me3, and H4K20me1, that occur after the *Xist*
- RNA “cloud” has formed and the Polycomb repressor complex 2 (PRC2) has been activated.
- These changes allow for the recruitment of epigenetic modulators, such as heterochromatin protein HPI, which binds H3K9me2 to cover the X and ensure its heterochromatinization.
- Hypoacetylation of histones H3 and H4 further contribute to this event, as does the insertion
- of the repressor histone variant macroH2A and the monoubiquitination of histone H2A.

